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Review

The potential application of avian egg antibodies with emphasis on immunotherapeutic and immunodiagnostic purpose

Teshager Dubie^{1*}, Tesfaye Sisay², Fikre Zeru¹, Mu-uz Gebru¹ and Yimer Muktar³

¹College of Veterinary Medicine, Samara University, P.O. Box 132, Samara, Ethiopia.

²Institute of Biotechnology, College of Natural and Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia.

³College of Veterinary Medicine, Haramaya University, P.O. Box 138, Dire Dawa, Ethiopia.

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Avian eggs present an ideal alternative antibody source to mammals as the immunoglobulin (IgY) in the chicken's blood is transported to the egg and accumulates in the egg yolk in large quantities. The existence of an immunoglobulin G (IgG)-like molecule in avian eggs, referred to as IgY, has been well documented, and extensive research has been carried out on its characterization, production and purification. Although it is the functional equivalent of mammalian IgG, the major serum antibody found in mammals IgY is structurally different, and has been found to exhibit several important differences when compared to mammalian antibodies, including its physicochemical properties and immunological capabilities. Recently, considerable research has focus seldom use of IgY as an alternative to mammalian antibodies for several applications, including immunotherapeutic applications, especially for the oral passive immunization against various bacteria and viruses. Much research has also been carried out on the use of IgY as a replacement for IgG in various immunodiagnostic and immunoaffinity purification purposes. The use of IgY offers several advantages over polyclonal antibodies produced in mammals, including providing a much more hygienic, cost efficient, convenient, humane and plentiful source of antigen-specific antibodies.

Key words: Avian, egg yolk antibody, immunodiagnostic, immunotherapeutic, IgY.

INTRODUCTION

The avian egg contains all the necessary nutrients and growth factors required for the developing embryo, including antibodies that are transported from the blood of the hen into the egg yolk to provide immunity to the chick (Yegani and Korver, 2010). The production of antibodies (Abs) in chickens and the extraction of specific

Abs from egg yolk (IgY Abs) are increasingly attracting the interest of the scientific community as demonstrated by the significant growth of the IgY literature. Avian eggs present an ideal alternative antibody source to mammals as the IgY in the chicken's blood is transported to the egg and accumulates in the egg yolk in large quantities.

*Corresponding author. E-mail: teshager.dubie@yahoo.com.

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Maternal antibody can be transferred from hens to the chicks either through the placenta, colostrum, milk, or egg (Grindstaff et al., 2003). Birds transmit maternal antibodies to their offspring by depositing the antibodies in the egg (Brambell, 1970). There are three classes of antibodies in chickens, namely Immunoglobulin IgY (IgG), IgA and IgM. Chicken IgA and IgM are similar to mammalian IgA and IgM in terms of molecular weight, structure, and immunoelectrophoretic mobility. In eggs, IgY is present predominantly in the egg yolk (Leslie and Clem, 1989) whereas IgA and IgM are present in the egg white as a result of mucosal secretion in the oviduct (Rose et al., 1994).

Hen eggs consist of approximately 9.5% egg shell (including shell membrane), 63% albumen, and 27.5% yolk. The main components are water (75%), proteins (12%), lipids (12%), as well as carbohydrates and minerals (1%) (Burley and Vadehra, 1989). The proteins are distributed throughout the egg with the majority found in the egg yolk and egg white, and a small proportion in the egg shell and shell membrane (Watkins, 1995). The lipids are found almost exclusively in the egg yolk, mainly in the form of lipoproteins (Burley and Vadehra, 1989). Several minerals have also been found in eggs, most of them in the eggshell. Carbohydrates are a minor egg component, present throughout the egg, both as free and conjugated forms, attached to proteins and lipids (Watkins, 1995).

IgY in avian egg has many applications in the medical and research fields, including in the areas such as diagnostics and proteomics. However, the most valuable and promising areas of IgY research is its use for passive immunization to treat and prevent human and animal diseases. Antibodies from eggs may have also many applications against microorganisms in humans and livestock or poultry (Gibbins, 1977). Serum antibodies of hyper-immunized hens are efficiently transferred and accumulated in the egg yolk (Fichtali et al., 1994). There are also efficient cation exchange chromatographic techniques for separating these antibodies from egg yolk. Coleman (1998) reported that antibodies from eggs can be effectively used to treat mastitis in dairy cows and may also have potential in treating human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS). Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies (Tizard, 2002). The chicken immune system has been studied for many years, and these studies have contributed substantially to the understanding of the fundamental concepts of immunology and the development of different immunoglobulin classes (Carlander et al., 1999). IgY is the major antibody in birds, reptiles and lungfish (Warr et al., 1995). In birds, the IgY is found mainly in blood and in the fluid fraction of the egg providing protection to newly hatched chick (Schade et al., 2005).

When animal welfare became more relevant in scientific studies, researchers began to seek alternatives to reduce the indiscriminate use of animals for research and diagnostic purposes. Although, IgY and IgG are sometimes used as synonyms in the scientific literature, the term IgY has become universally accepted based on its unique features (Tizard, 2002). Although functionally similar, there are several important differences between mammalian IgG and avian IgY (Sharma, 1997), and the use of avian antibodies offers many advantages over mammalian antibodies. The production of specific IgY against many different antigens has been studied, and its application as an immunotherapeutic agent including its use for the oral passive immunization against enteric pathogens has been extensively reported. Due to its distinctness from IgG, IgY has also been found to be advantageous in several techniques as well as in immunoaffinity purification, in many cases replacing IgG. Recently, the chicken has attracted considerable attention as an alternative source of antibodies. IgY is deposited in the egg yolk in large quantities (Janson et al., 1995), and it can be easily purified from the yolk by simple precipitation techniques, making chickens an ideal source for specific polyclonal antibodies (Gassmann et al., 1990).

Antibody purification involves selective enrichment or specific isolation of antibodies from serum (polyclonal antibodies), ascites fluid or cell culture supernatant of a hybridoma cell line (monoclonal antibodies). The need to develop effective, economical and rapid purification methods of monoclonal and polyclonal antibodies from a variety of biological fluids becomes imperative for *in vitro* or *in vivo* application. Antibody purification can be divided into two main groups: precipitation methods and chromatographic methods. Purification of immunoglobulin from mammalian blood is time-consuming and expensive. Today, hens are recognized as a convenient and inexpensive source of antibodies. It has been reported that the amount of immunoglobulin that can be yielded from one egg of an immunized hen is as much as that can be obtained from 300 ml of rabbit blood. Chicken egg yolk antibodies (IgY) have been applied successfully for scientific, diagnostic, prophylactic and therapeutic purposes. Because of the phylogenetic distance between birds and mammals, mammalian proteins are often more immunogenic in birds than in other mammals and antibody synthesis readily stimulated in hens (Bizhanov et al., 2004).

IgY and IgG egg yolk antibodies have been used in many diagnostic and biomarker discovery applications as a result of immunoreactivity difference. However, much research has focused on the use of IgY for passive immunization application. Passive immunization has recently become an even more attractive approach because of the emergence of new and drug resistant microorganisms, and individuals with impaired immune system who are unable to respond to conventional

Table 1. Comparisons of mammalian IgG and chicken IgY.

Animals	Mammals (IgG)	Chicken (IgY)
Source of antibodies	Blood serum	Egg yolk
Kind of antibodies	Polyclonal	Polyclonal
Antibody sampling	Bleeding	Collecting egg
Antibodies amount	200 mg/blood	100-150 mg/egg
Quantity of antibody	1400 mg	40,000 mg

Source: Schade et al. (1991).

vaccines. Passively administered antibodies have the ability to provide rapid and immediate protection; for example, against agents of bioterrorism (Casadevall et al., 2004). The reduction of antibiotics use in the livestock industry and increasing evidence that resistant organism may pass from animals to humans, resulting in infections that are harder to treat (Yegani and Korver, 2010).

Therefore, this paper aim to assess several aspects of avian immunoglobulins and the avian immune system, including the structure, production and purification of IgY, and to outline many current and potential applications of IgY, especially in the areas of immunotherapy and immunodiagnosics.

AVIAN EGG FORMATION

The hen's reproductive system is a very complex system that can produce an egg in 24 h. The formation of an egg involves the conversion of the feed into egg constituents through a number of intricate and highly coordinated steps as a storehouse of nutrients. The formation of an egg occurs in the ovary and oviduct. Although two sets of ovaries and oviducts are present during embryonic development only the left set fully develop in chickens. When the chicken becomes mature (about 150 days old), the ovary grows to about 7 g and rapidly increases to about 40 g (around 170 days old) (Burley and Vadehra, 1989). The mature ovary will have several follicles in different development stages at any one time and the largest follicle is the one to be ovulated to produce an egg firstly. Yolk constituents are synthesized in the liver and they are transported to the follicular walls in the blood. The follicle undergoes a rapid development during which most of the yolk is deposited 6 to 10 days prior to ovulation, when sufficient yolk has accumulated. The follicle in the ovary is ovulated into the oviduct where the yolk is enveloped in albumen and the shell. It takes 24 to 27 h for this development. In laying hens, the oviduct is 40 to 80 cm long with an average weight of 40 g, and consists of five regions, infundibulum, magnum, isthmus, uterus and vagina (Burley and Vadehra, 1989). The infundibulum is the top portion of the oviduct; with a broad funnel shaped anterior end (8 to 9 cm) and a narrow posterior end to receive the ovulated follicles

(Burley and Vadehra, 1989). An egg consists of the yolk (30 to 33%), albumen (~ 60%), and shell (9 to 12%) (Figure 1). The total solids content of egg yolk is generally around 50%, but can vary with the age of the hen and the storage of the shell eggs. The major constituents of the solid matter of yolk are proteins and lipids, present mainly in the form of lipoproteins (Li-Chan et al., 1995). Their relative amounts can be seen in Table 1. The yolk can be separated by high speedcentrifugation into sedimented granules and a clear fluid supernatant called plasma. Granules are composed of 70% α - and β -lipovitellins, 60% phosvitin, and 12% low-density lipoprotein. The plasma is divided into the low-density lipoprotein fraction (33%) and the water soluble fraction (WSF) (5%), which contains the livetins, which are lipid-free globular proteins, including g-livetin, also referred to as IgY (Li-Chan et al., 1995).

AVIAN EGG ANTIBODIES

Avian immune system

The chicken immune system consists of the bursa of fabricius, bone marrow, spleen, thymus, the harderian gland, lymph nodes, circulating lymphocytes, and various lymphoid tissues. The thymus serves as the primary lymphoid organ for T-cell differentiation while the antibody-synthesizing B-cells are produced in the bursa of fabricius (Carlander et al., 1999). The spleen is the centre for plasma cell proliferation and memory B-cells (Carlander et al., 1999). Previously, antibodies presently available for research, diagnostic and therapies were mostly mammalian monoclonal or polyclonal antibodies, but now a daychicken egg yolk antibodies (IgY) which has have been applied successfully for scientific, diagnostic, prophylactic and therapeutic purposes (Bizhanov et al., 2004). Chicken IgY is highly concentrated in egg yolk than it is in serum. The chicken is an excellent producer of antibodies. Even though avian IgY has been applied, it is under use according to some literature. This may be due to lack of information concerning the different methods and applications where IgY is more advantageous compared to the traditional mammalian IgG antibodies (Larsson et al., 1993). Avian

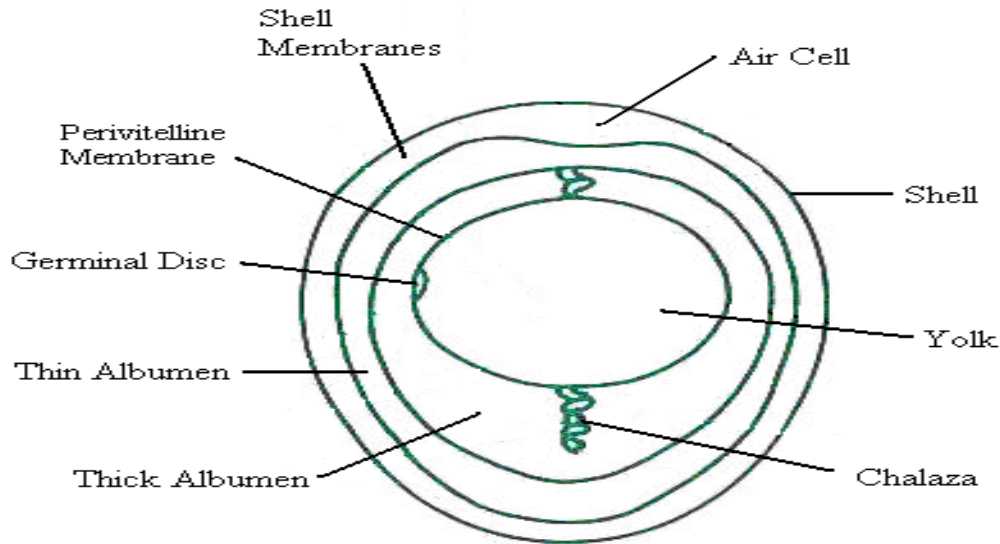


Figure 1. Formation of the hen's egg.

antibodies contain both heavy (H) and light (L) chains that are encoded by two unlinked loci. In the light chain locus there are only single gene segments each for the V and J regions. The heavy chain has only one segment each for V and J regions, and about 15 D segments (Sharma, 1997). Therefore, rearrangement contributes little diversity in chicken B-cells, in contrast to mammals, because there are only single gene segments for the V and J regions. Only the D segments serve to introduce a combinatorial factor of diversity (Reynaud et al., 1989). Birds instead attain antibody diversity using sequences of pseudo genes (25 for the light chain and around 100 for the heavy chain) in a process of gene conversion in which segments of pseudogenes are inserted into the V-region (Sharma, 1997). In this way, despite the fact that chickens have an extremely limited number of immunoglobulin genes, compared to mammals, they are capable of producing a wide range of immune responses and diverse antibody molecules (Sharma, 1997).

Biosynthesis

Three immunoglobulin classes have been shown to exist in chicken: IgA, IgM, and IgY. The IgA and IgM are similar to mammalian IgA and IgM. Chicken IgY is the functional equivalent of IgG, the major serum antibody found in mammals, and makes up about 75% of the total antibody population (Carlander et al., 2000). In mammals, the transfer of maternal antibodies can take place after birth, however in the chicken; the maternal antibodies must be transferred to the developing embryo aim to give acquired immunity to the chick (Sim et al., 2000). Antibody, specifically IgA and IgM, is secreted into the ripening egg follicle and is incorporated into the egg white

in the oviduct along with the egg albumen secretion. Serum IgY is selectively transferred to the yolk via a receptor on the surface of the yolk membrane which is specific for IgY translocation (Morrison et al., 2002). Egg white contains IgA and IgM at concentrations of around 0.15 and 0.7 mg/ml, respectively, whereas the yolk may contain from 5 to 25 mg/ml of IgY (Li et al., 1997). Mammalian equivalents of IgE and IgD have not been identified in chickens (Sharma, 1997).

Structure of immunoglobulin Y

The structure of IgY is significantly different from that of mammalian IgG even though there is similarity in their function (Carlander et al., 1999). IgY contains two heavy (H) and two light (L) chains and has a molecular mass of 180 kDa, larger than that of mammalian IgG (159 kDa). IgY possesses a larger molecular weight H chain (68 kDa) as compared to that from mammals (50 kDa). The H chain of IgG consists of four domains: the variable domain (VH) and three constant domains (C_V1, C_V2 and C_V3). The C_V1 domain is separated from C_V2 by a hinge region, which gives considerable elasticity to the Fab fragments. In contrast, the H chain of IgY does not have a hinge region, and possesses four constant domains (C_V1- C_V4) in addition to the variable domain. Sequence comparisons between IgG and IgY have shown that the C_V2 and C_V3 domains of IgG are closely related to the C_V3 and C_V4 domains, respectively, of IgY, while the equivalent of the C_V2 domain is absent in the IgG chain, having been replaced by the hinge region (Warr et al., 1995). The content of β -sheet structure in the constant domains of IgY has been reported to be lower than that of IgG, and the exibility between the C_V1 and C_V2 domains,

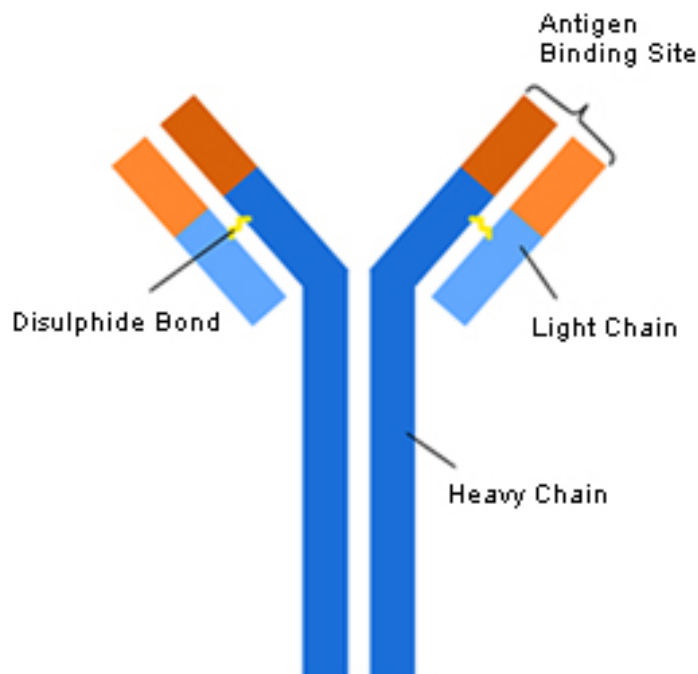


Figure 2. Structure of antibodies.

corresponding to the hinge region of IgG, is less than that of IgG (Shimizu et al., 1992). Unlike IgG, IgY has two additional Cys residues, Cys 331 and Cys 338, in the Cv2 Cv3 junction, which were likely to participate in the inter-chain disulfide linkages (Warr et al., 1995) (Figure 2).

Origin of immunoglobulin Y

Although IgM is the only universally distributed antibody and is believed to be the precursor for all immunoglobulin classes, current evidence suggests that IgY may have instead been the immediate progenitor of both IgG and IgE (Warr et al., 1995). The comparisons of IgY and IgG are listed in Table 1. It does not also have the ability to precipitate or agglutinate multivalent antigens unless at high salt concentrations (around 1.5 M), perhaps due to steric hindrance caused by the closely aligned Fab arms of the IgY molecule. High salt concentrations may serve to release the Fab arms, permitting agglutination.

Production and purification of immunoglobulin Y

Chickens can be used for antibody production throughout their entire egg laying period. Animals that are used for antibody production for more than three months should be given booster immunizations every other month to assure that the antibody titer remain high. Chickens can produce high avidity antibodies already after one immunization, compared to sheep whose avidity

becomes similar after four immunizations (Landon and Woolley, 1995). Chicken eggs present an ideal alternative antibody source to mammals, as the IgY in the chickens' blood is transported to the egg and accumulates in the egg yolk in large quantities. The amount of antigen specific antibodies of the total pool of antibodies in an egg has been reported to be up to 10 %. However, the actual amount of specific antibodies probably varies depending on the individual animal, immunization procedures and the immunogenicity of the antigen itself (Carroll and Thalley, 1990).

The major problem in isolating IgY from egg yolk is separating the lipoproteins from egg yolk prior to purification of the IgY (Kim et al., 1999). There are several methods of purification of IgY described. These IgY separation methods include: lipoprotein precipitation by polyethylene glycol (Svendson *et al.*, 1995), sodium dextran sulphate and natural gums such as xanthan gum (Akita and Nakai, 1993), and dextran blue (Bizhanov and Vyshniauskis, 2000), and sodium alginate (Hatta et al., 1990). Chang et al. (2000) recently reported the precipitation of over 90% of lipoproteins from yolk using l-carrageenan, sodium alginate, carboxymethyl cellulose, and pectin. Ion exchange chromatography has also been reported as a final step in IgY purification (Fichtali et al., 1993), as well as hydrophobic interaction chromatography (Hassl and Aspöck, 1988), immobilized metal ion affinity chromatography (Greene and Holt, 1997), thiophilic interaction chromatography (Hansen et al., 1998), affinity chromatography using alkaline conditions (Kuronen et al., 1997), and synthetic peptide

ligands, designed specifically for immobilizing antibodies (Verdoliva et al., 2000). As well, Erhard et al. (1996) described a method for the purification of mouse IgG subclass specific IgY using indirect affinity chromatography with protein G Sepharose (Deignan et al., 2000). The choice of the methods is a matter of yield and purity desired, final use of the IgY as well as material cost and labor skills. The best way to obtain antibodies is to purify them from the yolk. Several methods can be used, even for large-scale purification, of functionally active chicken antibodies from egg yolk. Over 100 mg of purified IgY can be obtained from a single egg and it is also possible to purify specific antibodies by affinity-chromatography (Akita and Nakai, 1998).

Physico-chemical properties

IgY and IgG differ not only in structure, but also in their stability to pH, heat, and proteolytic enzymes. Although the stability of both immunoglobulins was similar when subjected to alkaline conditions, IgY showed much less stability than that of rabbit IgG to acid denaturation. Shimizu et al. (1993) found that the activity of IgY was decreased by incubating at pH 3.5 or lower and completely lost at pH 3. The rabbit IgG antibodies, on the other hand, did not demonstrate a loss of activity as the unit of the pH decreased to by 2, and even then some activity still remained. Similar results were also observed by Hatta et al. (1993), using IgY produced against human rotavirus. Similarly, the IgY was significantly more sensitive to heating than the rabbit IgG. Shimizu et al. (1992) found that the activity of IgY was decreased by heating for 15 minutes at 70°C or higher, whereas that of the IgG did not decrease until 75 to 80°C or higher. Hatta et al. (1993) found, using differential scanning calorimetry (DSC), that the temperature corresponding to the maximum of denaturation endotherm (T_{max}) was 73.9°C for IgY and 77.0°C for IgG. Shimizu et al. (1994), however, described the addition of sugar to an IgY solution, and found high concentrations of sugar allowed the IgY to maintain activity when subjected to high heat (75 to 80°C), low pH (3), or high pressure (5000 kg/cm²). IgY, like IgG, has been found to be relatively resistant to trypsin and chymotrypsin digestion, but sensitive to pepsin digestion (Shimizu et al., 1988). Hatta et al. (1993) found that almost all of the IgY activity was lost following digestion with pepsin, however activity remained even after 8 h incubation with trypsin or chymotrypsin. Otani et al. (1991) found that IgY was, however, more susceptible to digestion with trypsin, chymotrypsin and pepsin than IgG. The proteolytic digestion of antibodies is a common technique, used to remove the cross-reacting Fc portion of the antibody molecule. Akita and Nakai (1993b) noted further differences between IgY and IgG, with the peptic digestion of IgY resulting in mainly monovalent Fab' fragments, while the peptic digestion of IgG yields the bi-

valent (F(ab')₂) fragments. The structural factors resulting in the stability differences of the two immunoglobulins are unknown, as immunoglobulins are large, complicated molecules, composed of heterogeneous polypeptides. Shimizu et al. (1992) predicted that the lower content of b structure in IgY may indicate that the conformation of IgY is more disordered and therefore less stable than mammalian IgG.

Advantages of immunoglobulin Y

The use of chickens for the production of polyclonal antibodies provides several advantages over the traditional method of producing antibodies in mammals. In contrast to mammalian serum, egg yolk contains only the single class of antibody, IgY, which can be easily purified from the yolk by simple precipitation techniques (Gassmann et al., 1990). The phylogenetic distance between chickens and mammals renders possible outcomes on the production of antibodies, in chickens, against highly conserved mammalian proteins, that would otherwise not be possible in mammals, and much less antigen is required to produce an efficient immune response (Larsson et al., 1988). Chicken antibodies will also recognize different epitopes than mammalian antibodies, giving access to a different antibody repertoire than with mammalian antibodies (Carlander et al., 1999). As well, the method of producing antibodies in hens is much less invasive, requiring only the collection of eggs, rather than the collection of blood, and is therefore less stressful on the animal (Schade et al., 1991), and sustained high titres in chickens reduce the need for frequent injections (Gassmann et al., 1990).

The animal care costs are also lower for the chicken compared to that for mammals, such as rabbits (Carlander et al., 2000). Hens therefore provide a more hygienic, cost efficient, convenient, and plentiful source of antibodies, as compared to the traditional method of obtaining antibodies from mammalian serum (Carlander et al., 2000). Nakai et al. (1994) estimated that the productivity of antibodies in hens is nearly 18 times greater than that by rabbits based on the weight of antibody produced per head. Because of the high yolk IgY concentrations, over 100 mg of IgY can be obtained from one egg (Akita and Nakai, 1992). A laying hen produces approximately 20 eggs per month; therefore, over 2 g of IgY per month may be obtained from a single chicken (Carlander et al., 1999). In the egg, IgY is stable for months, and once purified it may be stored for years in the cold (Larsson et al., 1993). As the industrial scale automated collection and separation of eggs is currently carried out, the large-scale production of specific IgY for immunotherapeutic purposes is feasible (Cotterill and McBee, 1995). Similarly, vaccination of chicken flocks has long been used to control avian infections (Sharma, 1999), making the injection of chickens required for large-

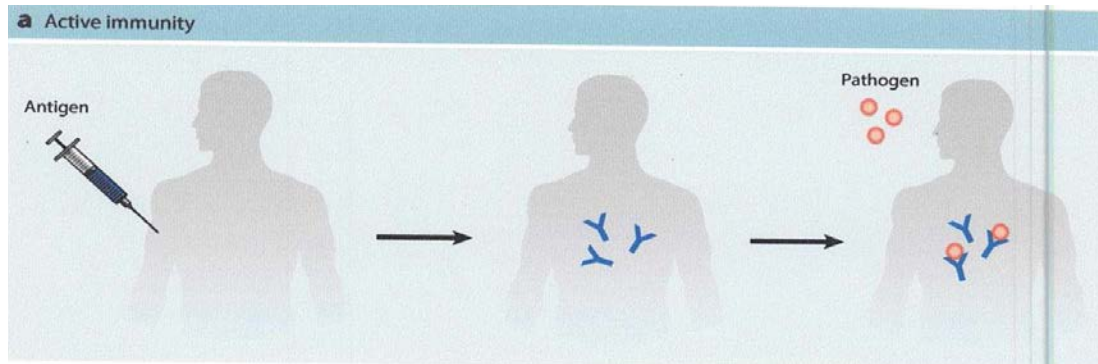


Figure 3. Active immunity involves immunizing, or vaccinating, an individual with an antigen to generate an adaptive response targeting the pathogen of interest.

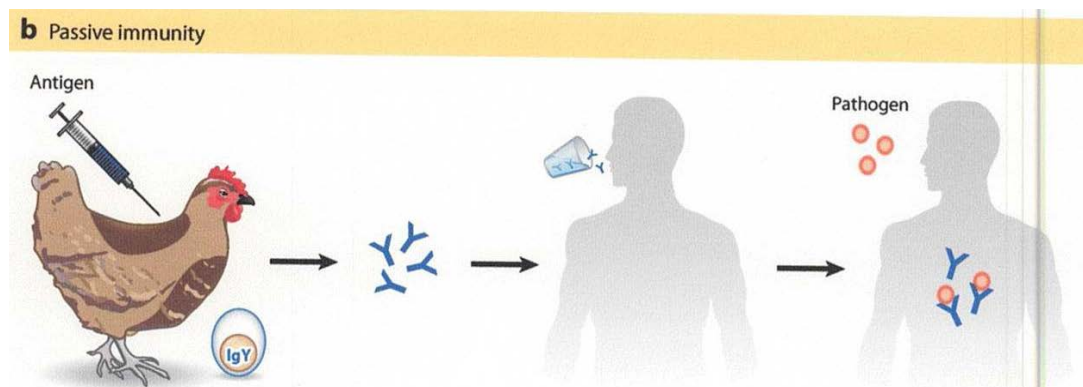


Figure 4. In passive immunization, antibodies are isolated from another source (example, the egg yolk of immunized hens) and administered to susceptible individuals to provide pathogen-specific immunity. Source: Baxter (2007).

scale antibody production also feasible.

Passive and active immunization of immunoglobulin Y

Active immunity refers to the process of exposing the individual to an antigen to generate an adaptive immune response. This response takes days or weeks to develop but may be long lasting. While, passive immunity refers to the process of providing preformed antibodies to protect against infections, and also provides immediate but short-lived protection lasting several weeks to three or four months at most (Baxter, 2007) (Figures 3 and 4).

THE POTENTIAL APPLICATIONS OF IMMUNOGLOBULIN Y

Immunotherapeutic applications of immunoglobulin Y
Passive immunization using specific antibodies is a

recent concept, which presents an attractive approach to establish passive immunity against pathogens in both humans and animals (Carlander et al., 2000). Previously, immunotherapy was carried out via the systemic or intravenous administration of specific antibodies for such applications as a targeting agent for cancer diagnosis and therapy, the inactivation of toxic substances including drugs and as passive immunotherapy for neoplastic or infectious diseases (Reilly et al., 1997). However, there has been increasing interest in the oral administration of specific antibodies for localized treatment of infections (Reilly et al., 1997). The increase in antibiotic-resistant bacteria and the desire to treat pathogens that do not respond to antibiotics such as viral pathogens, along with the escalating number of immune-compromised individuals has prompted much research into the administration of specific antibodies as an alternative to antibiotics and antimicrobial chemotherapy to treat infections. It is for this reason that much of the IgY research carried out has been with regard to immunotherapy (Carlander et al., 2000). Nowadays, there is pro-

gress to use chicken egg as source of antibodies for prevention and treatment of gut associated infections wherein, after immunization, the specific antibodies, otherwise, known as IgY are transported to the egg yolk and they can then be separated without scarifying the bird. Oral administration of IgY has been tried and found useful in treatment of human and animals against microbes. The potential applications of IgY for prevention and treatment of infections caused by pathogenic bacteria and viruses have been studied at length (Michael et al., 2010) and discussed.

Veterinary applications of immunoglobulin Y

Feed grade antibodies derived from the egg yolks of immunized hens have the advantage of being easily accessible, inexpensive and a rich source of polyclonal antibodies (Cook and Trott, 2010). Because of the ability of laying hens to produce large quantities of egg yolk antibodies on a relatively ongoing basis have been promoted and tested as potential feed grade prophylactic agents (Cook and Trott, 2010). They have been administered as potential inhibitors of the enzyme uricase to reduce nitrogen emissions in poultry due to the excess production of uric acid in the manure by microorganisms (Kim et al., 2013). The ability to generate specific antibodies in fairly large quantities has also proven advantageous for therapeutic prevention of microbial pathogen colonization. Incorporating feed grade egg yolk antibodies into animal diets has been examined extensively to attempt to limit pathogenic diarrhea causing *Escherichia coli* (*E.coli*) in swine, and limit *Salmonella* establishment in calves and mice, as well as *Campylobacter*, *Clostridium*, and *Salmonella* in poultry (Al-Adwani et al., 2013).

Egg yolk antibodies have also been developed for attempts to prevent establishment of food borne pathogens that commonly colonize food animals. *Campylobacter jejuni* is one of the major food borne disease causing microorganisms that also happens to be very well adapted to the ecological conditions prevalent in the poultry gastrointestinal tract (Pendleton et al., 2013). In an attempt to isolate antibodies that could limit *C. jejuni* colonization Al-Adwani et al. (2013) generated chicken egg-yolk-derived antibodies (IgY) in laying hens against the five different *C. jejuni* colonization-associated cell surface proteins. These proteins were produced in sufficient quantities by first expressing the respective protein in *E. coli* and subsequently purifying the proteins for intramuscular injection as a water-oil mixture in combination with Freund's complete adjuvant into *C. jejuni*-free laying hens. Eggs were collected up to 10 weeks post-immunization and egg yolks were lyophilized for eventual purification and quantization of specific egg yolk antibodies reactive to each of the *C. jejuni* proteins.

After characterizing specificity and reactivity of the

individual egg yolk antibodies generated against the specific cell surface proteins they demonstrated that several of these egg antibodies limited attachment of *C. jejuni* to chicken hepatocellular carcinoma cells and concluded that these were candidate egg yolk antibodies with potential to reduce *C. jejuni* colonization in chickens (Al-Aldawani et al., 2013).

Bovine rotavirus (BRV) is an important cause of diarrhea in newborn calves and local passive immunity is the most efficient protective strategies to control the disease (Vega et al., 2011). More recently, it was shown that anti-BRV IgY-containing yolk provided up to 80% protection against BRV-induced diarrhea in neonatal calves when compared with calves given non-immunized egg yolk suggesting that supplementing newborn calves' diets for the first 14 days of life with BRV-specific IgY may be a promising strategy to prevent BRV-related mortality (Vega et al., 2011). Diarrhea due to enterotoxigenic *E. coli* (ETEC) is a major health problem in humans and animals. IgY could be an alternative source of immunoglobulins for the prevention of ETEC infection as it has been found to inhibit the binding of *E. coli* to the intestinal mucosa (Jin et al., 1998). IgY raised against ETEC antigen has been administered orally to piglets and has offered a potential prophylactic and therapeutic approach for controlling ETEC-induced diarrhea (Marquardt et al., 1999). Marquardt et al. (1999), found out that the IgY titre was much higher when *E. coli* fimbrial antigen was used rather than the whole cell. Imberechts et al. (1997) raised IgY against *E. coli* F18ac fimbriae and in vitro adhesion tests demonstrated that the IgY inhibited attachment of F18ac positive *E. coli* to the intestinal mucosa. The anti-F18ab antibodies were also found to diminish diarrheal cases and death in animals infected with F18ac positive *E. coli*. Yokoyama et al. (1992) studied the passive protective effect of IgY against ETEC infection in neonatal piglets. IgY was administered to the piglets in milk three times a day for 2 days. Control piglets developed severe diarrhea within 12 h and 30% of the pigs died. In contrast, the pigs given IgY exhibited no sign of diarrhea 24 or 48 h after treatment (Marquardt et al., 1999). The passive protective effect of anti-ETEC IgY, in neonatal calves, against fatal enteric colibacillosis, has also been studied (Ikemori et al., 1992). Prevention of ETEC in rabbits through the oral administration of anti-ETEC IgY. Because the oral administration of anti-ETEC IgY has proven to be successful for the treatment of gastrointestinal infections of animals and also the clinical application of passive immunization of IgY against diarrhea is now being examined to prevent and treat ETEC infection in infants (O'Farrelly et al., 1992).

Salmonella enteritidis (SE) and *Salmonella typhimurium* (ST) are the main cause of outbreaks in human and infectious in chickens (Lee et al., 2002). Chalghoumi et al. (2009) found that IgY against the outer membrane proteins of SE and ST reduce salmonella spp.

adhesion to intestinal epithelial cells *in vitro*, which suggests that passive immunization with salmonella-specific IgY could be useful to prevent salmonella colonization in broiler chickens. Moreover, feeding chickens egg powder containing SE-specific antibodies was found to reduce fecal shedding, cecal colonization and the rate of salmonella-contaminated eggs in experimentally infected chickens (Rahimi et al., 2007).

Streptococcus mutans serotype c is thought to be the principal causative bacterium of dental caries in humans. The molecular pathogenesis of *S. mutans* associated dental caries involves a series of binding events that eventually lead to the accumulation of sufficient numbers of these carcinogenic bacteria to cause disease (Hamada and Slade, 1980). Chicken antibodies against *S. mutans* MT8148 serotype c or cell-associated glucosyltransferase were prepared and tested against dental caries (Chang et al., 1999). Consumption of a carcinogenic diet containing more than 2% IgY yolk powder resulted in significantly lower caries scores (Otake et al., 1991) and effective passive protection for the prevention of colonization of *S. mutans* in the oral cavity. It has also been reported that mouth rinse containing IgY specific to *S. mutans* was effective in preventing the dental plaque of humans *in vitro* and *in vivo* (Hatta et al., 1997).

Recently, Smith et al. (2001) produced IgY against the glucan binding protein B (GBP-B) of *S. mutans*. GBPs are believed to be involved in *S. mutans* biofilm development, and antibodies against GBP-B appear to have the potential to modulate infection and disease caused by *S. mutans*. Using a rat model of dental caries, they found that those rats treated with anti-GBP-B IgY displayed a decrease in *S. mutans* accumulation, as well as a decrease in the overall amount of dental caries, as compared to control rats. These studies indicate that IgY against *S. mutans*, or its components, may act to interfere with *S. mutans* accumulation and control plaque with the subsequent oral health problems associated with plaque accumulation (Smith et al., 2001).

In addition, specific IgY has been shown to be effective at preventing and treating several other pathogens. It was found that specific IgY was capable of preventing the pathogenesis of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Its use has also been suggested for passive protection of chicks against infectious bursal disease virus (IBDV) (Etteradossi et al., 1997), and for the protection against porcine epidemic virus (PEDV) in piglets (Kweon et al., 2000).

Applications of immunoglobulin Y in human medicine

IgY has been found to be effective against a number of human diseases causing pathogens both *in-vitro* and in laboratory animal studies and clinical settings. One of the most successful clinical applications of IgY has been in the prevention of *Pseudomonas aeruginosa* colonization

in the airways of cystic fibrosis (CF) in patients. In 2008, orphan drug designation was granted for IgY antibody against PA for the treatment of CF in humans by the European Medicines Agency. *P. aeruginosa* is the major cause of morbidity and mortality in CF patients and once a chronic infection has been established it is very difficult to eliminate, even with the use of antibiotics (Kollberg et al., 2003). Furthermore, there is increasing risk of developing antibiotic-resistant strains (Nilsson et al., 2008). In ongoing trials in CF patients, a mouth rinse containing purified anti-PA IgY given on a continuous basis could significantly reduce or prevent PA colonization, thereby reducing the need for antibiotics (Nilsson et al., 2008). These studies have shown that specific IgY is effective for immunotherapy for long treatment periods without negative side effects (Nilsson et al., 2007). The stability of the anti-PA IgY in the saliva of healthy individuals was also examined and antibody activity was shown to remain even after 8 h supporting the potential application of IgY for other localized infections such as the common cold and tonsillitis (Carlander et al., 2002). Another promising clinical application of IgY in human is the prevention of *Helicobacter pylori* infection. *H. pylori* is common cause of gastritis and gastric ulcers and the emergency of antibiotic-resistant strains has prompted the investigation into alternative treatment methods (DeLoney and Schiller, 2000). *In vitro* IgY against *H. pylori* reduced bacterial adhesion, growth and urease activity, and decreased *H. pylori* induced gastric mucosal injury and inflammation in an animal model. Because antibodies produced against whole-cell *H. pylori* might also cross-react with normal flora (Shin et al., 2003), the production and efficacy of IgY against immunodominant *H. pylori* proteins and peptides, including urease and urease-driven peptides (Nomura et al., 2005) and a 58 kDa highly reactive *H. pylori* antigen (Hp58) (Attallah et al., 2009), have been also examined. A functional drinking yogurt containing lactobacillus acidophilus and bifidobacterium species, supplemented with 1% antiurease IgY was produced commercially and given to volunteers testing positive for *H. pylori* (Horie et al., 2004).

Immunodiagnostic applications of immunoglobulin Y

The production came to be called "IgY Technology" (Warr et al., 1995), which is the internationally accepted term for describing the production and use of this antibody. Furthermore, the "European Centre for the Validation of Alternative Methods" (ECVAM) strongly recommends that yolk antibodies should be used as an alternative to mammalian antibodies for the animal welfare (Schade et al., 1996). The IgY can be harvested from the egg yolk instead of serum, thus making blood sampling outdated. The antibody productivity of an egg laying hen is greater than a similar sized mammal (Hau and Hendriksen, 2005)

and the IgY concentration in the serum of adult hens can reach approximately 5 to 7 mg/ml. As a laying hen produces approximately 20 eggs per month, over 2 grams of IgY can be isolated during this period corresponding approximately the IgY content of 300 ml of serum or 600 ml of blood. Only larger mammals can produce equal amounts of serum antibodies. Chicken antibodies, therefore, constitute a much less expensive vehicle for use in diagnostic purposes (Carlander, 2002).

The use of IgY can also be advantageous in immunological tests where the interference caused by IgG antibodies can be problematic, particularly, the sensitivity of the assay increases. One example is the rheumatoid factor (RF) that reacts with IgG from different mammalian species and also with mouse monoclonal antibodies (Carlander, 2002). RF is usually found in serum samples from patients with rheumatoid arthritis, but can also be found in patients with other diseases and even in 3 to 5% of healthy individuals. Interference by anti-IgG antibodies and antibody-binding substances have been demonstrated in approximately 40% of serum samples from healthy individuals in an immunoradiometric assay (Carlander, 2002).

Another important advantage arises from the phylogenetic distance and genetic background that distinguishes birds from mammals improving the likelihood that an immune response will be elicited against antigens or epitopes that may be non-immunogenic in mammals (Spillner et al., 2012). Due to the evolutionary distance between chicken and mammalian immunoglobulins, IgY recognizes more epitopes when the immunogen used is a mammalian protein which is highly conserved. This feature can result in amplification of the signal, emphasizing the advantages of using IgY over IgG as the first antibody in some types of immunological reactions (Carlander, 2002). It is a well-known concept that a stronger immune response is elicited when the distance between the antigen source and the immune system increases. It has also been shown that chicken antibodies have 3 to 5 times more affinity to antibodies of pigs than the rabbit IgG for signal amplification in immunological test (Olovsson and Larsson, 1993). The limited flexibility of the avian IgY may account for the inability to precipitate antigens at physiological salt concentrations (Warr et al., 1995). IgY and IgY(Fc) both possess two antigen-binding sites and should precipitate or agglutinate multivalent antigens but this does not always occur. Most chicken antibodies bind antigen strongly but display precipitating properties only at raised salt concentrations. Duck antibodies generally fail to exhibit efficient precipitation or agglutination reactions (Higgins, 1988). The non-precipitating duck antibodies do not acquire the ability to precipitate antigen at raised salt concentrations (Warr et al., 1995).

More recently chicken antibodies libraries have attracted scientific interest with increased reports on the isolation

of chicken derived antibody fragments. In other words, avian species utilize a unique mode of DNA recombination, named gene conversion, resulting in a large and diverse antibody repertoire upon antigen priming (Spillner et al., 2012). This could be exemplified by the development of a humanized chicken monoclonal anti-IL12 antibody (Nishibori et al., 2006). It is also important to keep in mind that recombinant technologies currently available can generate monoclonal IgY or IgY like antibodies from combinatorial libraries, sometimes without animal immunization (Spillner et al., 2012). Taking together, all these characteristics clearly show substantial advantages of IgY technology in many medical areas, especially for diagnosis. Specific chicken antibodies have been successfully raised against a wide variety of antigens including proteins, peptides, lipid hormones and carbohydrate components from viruses, bacteria, fungi, plants and animals (Schade et al., 1994). Several studies have also shown promising results in the development of techniques for immunodiagnostic using IgY, such as immunoassays tests to detect circulating antigen of *Schistosoma japonicum* (Cai et al., 2012), development of IgY antibodies against proteins of *Pythium insidiosum* (Rangel, 2010), use in antigen capture-ELISA (Veerasami et al., 2008).

Immunoglobulin Y in immunoaffinity chromatography

Immunoaffinity chromatography involves the isolation and purification of target molecules using immobilized antibodies directed against the target molecule. Due to the highly specific nature of the antibody-antigen interaction, immunoaffinity chromatography allows the purification of specific molecules from complex starting materials. The widespread use of this process in large scale, however, has been limited by the high cost of the technique and parameters relating to the production of antibody and the efficiency of immobilization (Li-Chan, 2000). Immobilized yolk antibodies have been used for the isolation of value-added proteins from dairy products, including the purification of lactoferrin (Li-Chan et al., 1998) and the isolation and separation of IgG subclasses from colostrum, milk and cheese whey (Akita and Li-Chan, 1998). Although IgY is more sensitive to low pH than IgG, Akita and Li-Chan (1998) reported that using standard affinity chromatography conditions (that is, elution at low pH), an IgY immunoaffinity column was stable and could be reused over 50 times without significant decreases in binding capacity. Alternative eluents have been examined, including highly alkaline conditions (Kuronen et al., 1997) and high concentrations of guanidine hydrochloride (Otani et al., 1991). To extend the use of IgY immunoaffinity columns, Kim et al. (1999) also examined the reusability of avidin-biotinylated IgY columns, in which biotinylated IgY is held by strong non-covalent interaction on columns containing immobilized

avidin. A number of other applications using IgY immunoaffinity columns have been described for the purification of biological molecules from human serum, including the purification of tetrachlorodibenzo-pdioxin (Shelver et al., 1998), prekallikrein (Burger et al., 1986), and human alpha-2 antiplasmin (Lee et al., 1997).

Other applications of immunoglobulin Y

It has been estimated that 1.7 million people are bitten or stung by venomous snakes, scorpions, jellyfish, or spiders each year, resulting in 40,000 to 50,000 fatalities. The most widely used treatment of envenomation is the use of specific anti-venoms to neutralize the toxic and potentially lethal effects of the venom. Chicken anti-venom IgY has been produced, and was found to have a higher bioactivity than anti-venoms raised in horses (Almeida et al., 1998). IgY also has a lower likelihood of producing significant clinical side effects, such as serum sickness and anaphylactic shock, which can occur upon administration of mammalian serum proteins (Larsson et al., 1993). Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases, which are an increasing burden to hospitals and society in terms of the cost of medication and treatment, and time lost due to illness (Hay and Hay, 1992). Standard medical care for these diseases includes anti-inflammatory drugs, immunosuppressants, and antibiotics, but their use is limited by side effects, immunosuppression, and incomplete efficacy. Immunotherapy using monoclonal mouse antibodies directed against tumor necrosis factor (TNF) has been approved for use, however it can be costly and adverse side effects have been reported in patients receiving systemic anti-TNF therapy (Sandborn and Hanauer, 1999). Recently, Worledge et al. (2000) reported that anti-TNF antibodies produced in chickens were capable of effectively treating acute and chronic phases of colitis in rats, and were also found to neutralize the treatment of inflammatory bowel disease in humans in the future. Human TNF *in vitro* indicates its possible use for the treatment of inflammatory bowel disease in humans in the future.

CONCLUSION

Chickens like mammals are capable of producing antigen specific antibodies IgY which functions are similar to IgG in response to an antigenic stimulus. It was not until recently, however, the particular immunological properties of IgY were recognized and IgY began replacing mammalian antibodies in such applications as immunodiagnostic assays, immunotherapeutics and affinity purification techniques. Yolk antibodies do not activate the mammalian complement system or interact with mammalian Fc receptors that could mediate inflammatory response in the gastrointestinal tract. As these

immunotherapeutic applications often require the continuous or frequent administration of antibodies, large quantities are required. IgY is, therefore, the ideal choice for the production of large quantities of conveniently purified antibodies. The use of IgY is also cost-effective with IgY costing less than \$10 per gram compared to IgG which can cost up to \$20 000 per gram. This technology will allow for new potential applications of IgY in medicine, public health, veterinary medicine and food safety. Chickens are useful for the production of specific IgY, and also needs to demonstrate the deposition of recombinant human antibodies into the egg yolk of transgenic chickens suggesting an extension of the production of specific IgY in eggs.

RECOMMENDATIONS

1. More research should be carried out on the potential methods of production and application of egg yolk antibodies.
2. To increase the use of IgY, techniques for both direct and indirect labelling must be optimized.
3. When antibodies are to be used for therapeutic purposes, the use of free from specific pathogens chicken is compulsory.
4. It is preferable to immunize chickens before they begin to produce egg, because the stresses induced by handling them have an adverse effect on egg production.
5. Further study on the application of IgY in immunotherapeutic and diagnostic purposes should be undertaken.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Full Length Research Paper

Correlation between age, weight, scrotal circumference and the testicular and epididymal parameters of Red Sokoto bucks

O. S. Ajani*, M. O. Oyeyemi and Olusoji J. Moyinoluwa

Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

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The effect of age, weight and scrotal circumference on the testicular and epididymal parameters was studied in the Red Sokoto bucks. Thirty-six testes from Red Sokoto bucks were used for this study. The animal's age, weight, and scrotal circumference were determined and correlated with testicular weight, testicular diameter, testicular length, epididymal length, gonadal and extra-gonadal sperm motility and viability. All parameters were correlated positively and negatively at various instances of $p < 0.01$ and $p < 0.05$. The sperm morphological abnormalities showed that tailless head (primary abnormality), headless tail (secondary abnormality), bent tail (secondary abnormality), curved tail (secondary abnormality), bent mid-piece (tertiary abnormality) and curved mid-piece (secondary abnormality), total sperm abnormalities and total cell count were found to be significant at $p < 0.01$. It was concluded that the age, weight and scrotal circumference of Red Sokoto buck were positively correlated to its testicular and epididymal parameters and therefore are potentially useful in the evaluation of the breeding soundness of the bucks.

Key words: Epididymis, Red Sokoto bucks, scrotal circumference, testes, weight.

INTRODUCTION

Goats are important domestic farm animals in the world as a source of meat, milk, skin and wool (Onakpa et al., 2010). In Nigeria, it has been estimated that there are about 34.5 million goats and this population makes it the second most important livestock species (Onakpa et al., 2010).

Three main varieties of goats are recognized in Nigeria: The Sahel, Desert or West African long-legged goat, the Red Sokoto goat and the West African Dwarf goat (Onakpa et al., 2010). The Red Sokoto goat is also called

Maradi, Red skin, Sokoto Red, Katsina Light Brown, Mambilla, Bornu White or Damagian Dapple Grey. They are mainly distributed in Northern Nigeria (Sokoto and Kano States) and Southern Nigeria where the climate is semi-arid with a single rainfall season of 4 to 6 months. They are owned by the Hausa speaking agricultural tribes (Wilson, 1984).

Although Red Sokoto goats are known to breed all year round, their fertility characteristics have not been fully documented to facilitate effective genetic improvement by

*Corresponding author. E-mail: os.ajani@mail.ui.edu.ng.

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selection and crossbreeding at all levels of production. In the male for instance, there is the need to establish measurable criteria for judging breeding soundness and guiding selection of males for breeding. These criteria include scrotal measurements, libido and semen quality tests (Michelson et al., 1981; Ogwuegbu et al., 1985) and the relationships between them.

Biometric parameters, such as scrotal circumference (SC), testicular weight (TW) and testicular length (TL), are essential measurements in the andrological evaluation of a breeding animal. Among these parameters, SC is used most often because it is easy to measure and displays a high correlation with body weight and reproductive capacity (libido), particularly sperm production (Brito et al., 2004). While the biometric data related to SC help define the reproductive parameters for a species, SC alone should not be used for the selection of breeders. Rather, a complete andrological evaluation (a breeding soundness examination), including an evaluation of semen quality, should be performed to certify the reproductive capacity of a male (Ohashi et al., 2007).

Some studies have shown the reproductive parameters of the Red Sokoto goat, but with none correlating the age, weight and scrotal circumference with the testicular and epididymal parameters. This study is therefore aimed at investigating the correlation of age, weight, scrotal circumference on the left and right testicular and epididymal parameters of Red Sokoto bucks.

MATERIALS AND METHODS

Experimental animals and sample collection

Thirty-six testes were collected from Red Sokoto bucks slaughtered at Bodija abattoir located in Ibadan North Local Government Area of Oyo State, Nigeria on geographic grid reference of longitude 3°5N and latitude 7°20 N. The body weights, scrotal circumference and ages of the animals were taken prior to slaughter. The intra-scrotal testes were maintained at a warm condition (37°C), immediately the animals were slaughtered and transferred to the laboratory.

Testicular and epididymal biometrics

The testes collected were separated from the epididymis and weighed individually. The right and left epididymides were also weighed after trimming off the body of the testes. The testicular circumference, testicular lengths and epididymal lengths were measured using a flexible metric tape and recorded.

Semen collection

Semen samples were collected from the body of the testes and epididymis through an incision made with a scalpel blade. The semen samples were analysed to determine the percentage sperm motility, viability and morphological characteristics as described by Zemjanis (1977).

Percentage motility

Percentage motility was evaluated with a drop of semen with drop of 2.9% buffered sodium citrate on a warm glass slide covered with a glass slip and viewed at a magnification of $\times 40$. Only sperm cells moving in a unidirectional motion were included in the motility rating, while sperm cells moving in circles, in backward direction or pendulating movement were excluded.

Percentage viability

Percentage viability was done by staining one drop of semen and one drop of warm Eosin-Nigrosin stain on a warm slide. A thin smear was then made of mixture of semen and stain. The smear was air dried and observed under the microscope. The ratio of the *in vitro* dead sperm cells was observed and it is based upon the principle of Eosin penetrating and staining the dead autolysing sperm cells whereas viable sperm repel the stain (Zemjanis, 1977).

Sperm morphology

A drop of semen was placed with two drops of Wells and Awa stain. The semen and stain were thoroughly mixed together, and a smear was made on another slide. The smear was dried and observed under light microscope, starting from lower power magnification to high magnification.

The presence of abnormal cells, out of at least 600 sperm cells from several fields on the slide was noted and their total percentage was estimated.

Data analysis

Simple correlation was calculated for some testicular parameters. Paired comparisons were done using students "t" test for sperm characteristics. Analysis of variance (One-way ANOVA) was used to compare the mean values of the testicular and epididymal parameters.

RESULTS

It was observed that both the right and left testicular parameters were positively correlated at 0.470 ($P < 0.01$), with the weight and scrotal circumference of the bucks. The weight and testicular weight of the bucks were positively correlated at 0.507 ($P < 0.01$), the scrotal circumference and testicular weight of the bucks were positively correlated at 0.781 ($P < 0.01$), the scrotal circumference and the testicular diameter of the bucks were positively correlated at 0.544 ($p < 0.01$), the scrotal circumference and the epididymal length of the bucks were positively correlated at 0.521 ($p < 0.01$), the testicular weight and testicular diameter of the bucks were positively correlated at 0.898 ($p < 0.01$), the testicular weight and the testicular length of the bucks were positively correlated at 0.363 ($p < 0.05$), the testicular weight and the epididymal length of the bucks were positively correlated at 0.635 ($p < 0.01$), the testicular diameter and the testicular length of the

Table 1. Descriptive statistics for the right and left testes and epididymides of Red Sokoto bucks.

Parameter	Mean ± Standard deviation (right)	Mean ± Standard deviation (left)	Mean ± Standard deviation (right and left)
Age (months)	44.7 ± 9.9	-	-
Weight (kg)	12.7 ± 2.3	-	-
Scrotal circumference (cm)	17.5 ± 1.4	-	-
Testicular weight (g)	52.6 ± 10.4	52.1 ± 9.6	52.3 ± 9.9
Testicular diameter (cm)	10.9 ± 1.0	11.0 ± 0.8	10.9 ± 0.9
Testicular length (cm)	5.1 ± 0.6	5.1 ± 0.8	5.1 ± 0.7
Epididymal length (cm)	8.0 ± 1.0	8.0 ± 1.0	8.0 ± 1.0
Gonadal motility (%)	24.4 ± 19.5	18.3 ± 17.6	21.4 ± 18.5
Gonadal sperm viability	86.3 ± 5.6	87.7 ± 6.0	87.0 ± 5.8
Extra-gonadal motility (%)	54.2 ± 31.4	56.1 ± 32.3	60.1 ± 34.2
Extra-gonadal sperm viability	94.6 ± 2.8	94.8 ± 4.1	94.7 ± 3.4

Means are not significantly different at $P < 0.05$.

Table 2. Correlations of age, weight, scrotal circumference, testicular weight, testicular diameter, testicular length, epididymal length, Gonadal and extra-gonadal sperm motility and viability of Red Sokoto buck.

Parameter	A	B	C	D	E	F	G	H	I	J	K
A	1	-0.322	0.085	0.256	0.287	0.153	0.197	-0.352*	0.119	0.044	0.152
B	-0.322	1	0.470**	0.507**	0.390*	0.188	0.333*	0.370*	-0.097	0.286	0.275
C	0.085	0.470**	1	0.781**	0.544**	0.232	0.521**	0.039	-0.137	-0.168	0.090
D	0.256	0.507**	0.781**	1	0.898**	0.363*	0.635**	0.084	-0.006	-0.109	0.036
E	0.287	0.390*	0.544**	0.898**	1	0.395*	0.514**	0.007	0.121	-0.185	0.056
F	0.153	0.188	0.232	0.363*	0.395*	1	0.696**	-0.244	-0.183	-0.128	-0.115
G	0.197	0.333*	0.521**	0.635**	0.514**	0.696**	1	-0.090	-0.312	-0.054	-0.040
H	-0.352*	0.370*	0.039	0.084	0.007	-0.244	-0.090	1	0.163	0.322	0.325
I	0.119	-0.097	-0.137	-0.006	0.121	-0.183	-0.312	0.163	1	0.003	0.419*
J	0.044	0.286	-0.168	-0.109	-0.185	-0.128	-0.054	0.322	0.003	1	0.278
K	0.152	0.275	0.090	0.036	0.056	-0.115	-0.040	0.325	0.419*	0.278	1

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level. A: Age (months), B: Weight (kg), C: Scrotal circumference (cm), D: Testicular weight (g), E: Testicular diameter (cm), F: Testicular length (cm), G: Epididymal length (cm), H: Gonadal sperm motility (%), I: Gonadal live-dead ratio (%), J: Extra-gonadal motility (%), K: Extra-gonadal live-dead ratio (%).

bucks were positively correlated at 0.395 ($p < 0.05$), the testicular diameter and the epididymal length of the bucks were positively correlated at 0.514 ($p < 0.01$), the testicular length and the epididymal length of the bucks were positively correlated at 0.696 ($p < 0.01$), the epididymal length and the gonadal sperm viability were negatively correlated at 0.312 ($p < 0.05$), the gonadal sperm viability and the extra-gonadal sperm viability were positively correlated at 0.419 ($p < 0.05$) (Tables 1 and 2).

The sperm morphological abnormalities showed that tail-less head (primary abnormality), headless tail (secondary abnormality), bent tail (secondary abnormality), curved tail (secondary abnormality), bent mid-piece (tertiary abnormality) and curved mid-piece (secondary abnormality), total sperm abnormalities and total cellcount were found to be significant at $p < 0.01$ in

the left testis compared to the right (Table 3).

DISCUSSION

It was observed in this study that as the weight of the animal increased, the scrotal circumference, testicular weight, testicular diameter, epididymal length and gonadal sperm motility also increased. As the testicular weight increased, the testicular length also increased. This is in agreement with the report of Raji and Njidda (2014) which stated that testicular weights have a high correlation with sperm reserves in the testes and epididymis and this is a direct reflection of testicular integrity for sperm production. Testicular weight has also been reported to highly correlate positively with body

Table 3. Testicular sperm morphological abnormalities of Red Sokoto bucks.

Parameter	Sample	Mean \pm Standard deviation
Tail-less head	Right	1.8 \pm 1.6
	Left	4.5 \pm 2.5*
Headless tail	Right	1.7 \pm 1.4
	Left	3.2 \pm 1.9*
Rudimentary tail	Right	1.9 \pm 2.6
	Left	3.1 \pm 2.3
Bent tail	Right	5.0 \pm 4.5
	Left	8.8 \pm 5.9*
Curved tail	Right	8.5 \pm 4.2
	Left	13.4 \pm 4.5*
Bent mid-piece	Right	2.8 \pm 1.9
	Left	5.0 \pm 2.3*
curved mid-piece	Right	4.6 \pm 2.0
	Left	6.7 \pm 3.2*
Looped tail	Right	1.1 \pm 0.9
	Left	1.8 \pm 1.9
Coiled tail	Right	0.5 \pm 0.8
	Left	0.6 \pm 1.1
Twin head	Right	0.1 \pm 0.3
	Left	0.8 \pm 2.0
Total abnormal cells	Right	27.4 \pm 15.2
	Left	47.8 \pm 17.5*
Total count	Right	235.7 \pm 196.0
	Left	674.1 \pm 149.8*

*Mean values are significant at the 0.01 level.

weight (Butswat and Zaharaddeen, 1998). There was a high correlation ($P < 0.01$) between the testicular weight and scrotal circumference. This conforms to the report of Willet and Ohm (1957). There was also correlation ($P < 0.01$) between testicular diameter and testicular weight. This also agrees with the report of Land and Carr (1975).

The values of the left and right testicular weights obtained from the present study were similar though it was observed that the right testis was heavier than the left testis. However, the values obtained were higher to

that reported by Raji and Njidda (2014) but similar to that reported by Raji et al. (2008) who reported value of 55.00 g for Red Sokoto bucks of age less than one year. The right testis being heavier than the left testis is not in agreement with the reports of Raji and Njidda (2014) in which the left testis was found to be heavier than the right testis. The epididymal length observed was lower than that reported by Raji and Njidda (2014). These contrasts might be attributed to differences in age, system of management and level of nutrition of the experimental animals used in this study.

The presence of abnormal forms of spermatozoa in this study is consistent with the report of Moss et al. (1979) that a number of abnormal forms are normally encountered in all ejaculates and that their presence in large numbers is often associated with impaired. The left testis fertility and epididymis had significantly increased the number of sperm abnormalities. This supports the report of Dunn (1980).

Conclusion

Age, weight and scrotal circumference of Red Sokoto buck were positively correlated to its testicular and epididymal parameters and therefore are potentially useful in the evaluation of the breeding soundness of the Red Sokoto bucks. Thus, it is therefore recommended that the age, weight and scrotal circumference of animals should be part of breeding soundness examination in the Red Sokoto bucks.

Conflict of interests

The authors declare that they have no conflicts of interest.

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Full Length Research Paper

Abattoir characteristics and seroprevalence of bovine brucellosis in cattle slaughtered at Bodija Municipal Abattoir, Ibadan, Nigeria

Dauda Garba Bwala^{1,3*}, Cheryl McCrindle², Folorunso Oludayo Fasina¹ and Ighodalo Ijagbone⁴

¹Production Animal Studies Department, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa.

²Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa.

³National Veterinary Research Institute, Vom 930001, Nigeria.

⁴Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria.

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***Brucella abortus* infection in humans in Nigeria has been recorded as a cause of febrile disease. In Nigeria, the transhumance (Fulani nomadic) husbandry system is the most common cattle farming system with about 95% of all the country's cattle population produced under this husbandry system. About 75% of all slaughtered cattle are processed in government-approved abattoirs. In view of the aforementioned, this abattoir can give a fair representation for a surveillance study of the Nigerian cattle population. 220 cattle were selected on arrival using systematic random sampling from a total slaughter population of 17,912 cattle, and were chosen over a 10-week period. Sixty-three percent (63.2%) of all slaughtered animals were cows, and only 4% were under 18 months (two-tooth). The indigenous breeds predominated and individual seroprevalence of *B. abortus* was estimated at 5.45% (n=12) using the Rose Bengal plate test. Currently, no safety measures is in place for abattoir workers and pre-slaughter monitoring for positive animals is lacking. Certain measures were suggested to reduce the zoonotic risk of human brucellosis from the slaughter process.**

Key words: Abattoir, bovine, brucellosis, Nigeria, seroprevalence.

INTRODUCTION

Bovine brucellosis is recognized as an important potential zoonoses in developing countries (McDermott et al., 2013; Ducrotoy et al., 2014). Recently, the World Health Organization has declared brucellosis to be a significant re-emerging zoonoses (World Health Organization [WHO], 2004; Seleem et al., 2010).

Bovine brucellosis was initially reported in 1927 but first recorded in Nigeria around 1928 (Ducrotoy et al., 2014), while the first case in Southwest Nigeria was reported in 1965 (Ducrotoy et al., 2014). Prevalence of bovine brucellosis between 0.2 and 80% across the different regions of Nigeria as well as between herds have been

*Corresponding author. E-mail: dgbwala@yahoo.com. Tel: +27 12 529 8466. Fax: +27 12 529 8306.

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reported by various authors and summarized by Ducrotoy et al. (2014), while institutional and abattoir prevalence between 3.7 and 48.8% have been reported in the Southern part of Nigeria (Cadmus et al., 2010, 2013). An earlier review of data on bovine brucellosis by the Food and Agriculture Organization of the United Nations (FAO) in Nigeria between 1974 and 1991 have also reported an individual seroprevalence at the abattoirs that ranged between 6.3 and 15.0% using the Rose Bengal plate test (RBPT) and 1.5 and 14.8% using the serum agglutination test (SAT) (Mangen et al., 2002).

Several cases of human brucellosis have been reported in Nigeria as summarized by Ducrotoy et al. (2014), but the first laboratory confirmed the case was recorded in 1941 (Ducrotoy et al., 2014). Although cases of human brucellosis are grossly underreported since it causes minimal mortality, it appears to be a cause of human febrile disease in Nigeria as in most African countries (Pappas et al., 2006). Several serological investigations for animal-originated human brucellosis in Nigeria have been carried out and prevalence of between 0 and 74% has been reported (Ducrotoy et al., 2014). However, most of the prevalence figures do not necessarily represent active disease, but only confirms exposure to *Brucella* as both RBPT and SAT tests which are routinely used to detect only agglutinating antibodies (Ducrotoy et al., 2014). Nevertheless, a number of researchers (Ofukwu et al., 2007; Diaz et al., 2011) that carried out seroprevalence studies for bovine brucellosis in human patients presenting with acute febrile illness or pyrexia of unknown origin (PUO) in Nigeria have suggested that brucellosis should always be considered when treating human patients with acute febrile reactions or symptoms.

Despite the evidences of brucellosis in human and animals (Diaz et al., 2011; Godfroid et al., 2011; Dean et al., 2012), particularly in Nigeria and the risks posed to abattoir workers, most of the studies done on brucellosis in the Bodija abattoir (Cadmus et al., 2010) did not assess the situations of the abattoir. In addition, Bodija abattoir is one of the largest abattoir in the Southwestern region with its attendant crowd and it receives cattle and other animals from different regions across the country for slaughter for human consumption. In view of the aforementioned, the abattoir is a potential source of infection to workers and visitors to the abattoir. The aim of this study therefore was to describe the abattoir activities and estimate the seroprevalence of bovine brucellosis in cattle presented for slaughter at the Bodija Municipal abattoir, in order to estimate the possible risks of zoonotic transfer to abattoir workers.

MATERIALS AND METHODS

Study area

Bodija is located in Ibadan North Local Government (Latitude 7° 24'40"N; Longitude 3° 35' 24"E), and it has a large, poorly organized

market where goods and services are traded daily as well as an associated abattoir. After the Oko Oba Abattoir in Lagos, Bodija Abattoir in Ibadan is the second largest abattoir in South-West Nigeria. Cattle, both locally raised within Nigeria and trade cattle from across the countries north of Nigeria are moved and transported mainly from the northern parts of the country towards the south especially to Lagos and Ibadan where they are slaughtered daily in thousands for consumption. Ibadan (the largest city in West Africa) acts as a distribution network and a market for a large percentage of these cattle. Specifically, the Bodija Municipal Abattoir receives cattle from different parts of Nigeria and even beyond the Nigerian borders and will therefore be suitable to do representative seromonitoring of *Brucella abortus* in slaughtering cattle in Southwest Nigeria and assess the likelihood of zoonotic implications to abattoir workers.

Sampling frame

A total of 220 animals were sampled using systematic random sampling from a total slaughter population of 17,912 animals over a period of 10 weeks (Table 1). The calculation of sample size was done using Survey Toolbox Version 1.04 (Cameron, 1999). It was assumed that all animals irrespective of age and sex were equally exposed to the risk of *Brucella* species, the sample frequencies were normally distributed and that the test protocol gave a valid true specificity and sensitivity as previously calculated.

Blood collection

Blood samples were collected in sterile non EDTA coated vacutainer tubes/bottles directly from the jugular veins of cattle (n=220) prior to slaughter, over a period of ten weeks. The age, sex, breeds and body condition scores of sampled cattle were also recorded after they were sampled as described by Marston (2005). The activities of the butchers and other abattoir workers ranging from when the animals were brought in from either the lairage or market to the abattoir to the final movement of the finished product (meat) were observed. Workers were similarly observed for apparent states of health, pace of work, swelling and other behavioural signs/symptoms indicative of human brucellosis.

The blood samples were allowed to clot in a slanting position, centrifuged for 10 min at 1500 rpm and sera were decanted into sterile Bijou bottles. A total of 220 sera were decanted for serological evaluation. The standardized but simple RBPT was performed at room temperature (approximately 25°C) on all samples (OIE, 2012). Briefly described, a drop (approximately 30 µl) of each serum was placed on a clean white porcelain tile. 30 µl of *B. abortus* RBPT antigen (VLA, Weybridge) was placed beside each drop using a clean Pasteur pipette. These were mixed together using sterile applicator sticks and the mixtures were rocked for 4 min. The plates were observed for formation of distinct pink granules (agglutination) and results were recorded as positive or negative. All sera were tested using this procedure. The graded positive and negative controls were done using reference sera from VLA, Weybridge.

Statistical analyses were performed using data entered into Microsoft Excel® Spreadsheet and calculated using the Student T-Test.

RESULTS

Abattoir activities

Ante-mortem inspection of animals was rarely done thoroughly and most aspects of the bleeding, removal of internal organs and flying

were done with the carcass on the abattoir floors contaminated with blood and intestinal contents. Numerous cases of manhandling and cruelty to animals were observed prior to slaughter and pre-slaughter stunning of cattle was never done. Most (>50%) of the animals were poorly bled leading to flushing and poor meat quality. No protective clothing was used by butchers and blood meal processors move about the abattoir freely to collect blood while animals are being slaughtered. Clean potable water was also in short supply in the abattoir and the entire slaughter process was carried out using a single bowl of water of less than 50 L per animal. The abattoir was almost always over-crowded due to the activity of touts, slaughter hands and blood meal producers.

Sampling result

In general, 17,912 cattle were slaughtered during the 10-weeks study period of which 6,596 (36.8%) and 11,316 (63.2%) were bulls and cows, respectively. Of the total 220 cattle sampled, 87 (39.54%), 92 (41.82%) and 41 (18.64%) were of good, fair and poor conditions, respectively (Table 1). Eighty eight (40%) of the cattle sampled were of the White Fulani breed, followed by Red Bororo with 68 (30.9%) and Adamawa Gudali having the least number of 5 (2.3%). In addition, 112 (50.9%) and 108 (49.1%) of the 220 cattle sampled were females and males, respectively, while 211 (95.9%) were above or greater than a year of age (Table 2). The gender distributions of slaughtered cattle sampled at the Bodija Municipal abattoir are as shown in Table 2. All the cattle presented for slaughter were indigenous breeds and they came primarily from transhumance and sedentary pasture-fed farming systems. No feedlot cattle was presented for slaughter and as such, majority (95.9%) of the slaughtered animals were adult, not steers under the age of two years, as practiced in developed countries. Serological investigation for bovine brucellosis in cattle slaughtered using the RBPT revealed a seroprevalence of 5.45% ($n=12$, $P < 0.0001$) (Table 3). These 12 animals include 5 (2.27%) mature bulls and 7 (3.18%) adult cows; 3 of which were in apparently good condition, 5 in fair condition and 4 cattle in poor condition.

DISCUSSION

Our study population was almost or wholly non-vaccinated cattle population from different areas of Nigeria since vaccination against brucellosis is not routinely practiced and the work has revealed some critical but important observations on the status of abattoir slaughter in Bodija Abattoir, Ibadan, Nigeria. We are aware that few cases of vaccination may have occurred but it is difficult to establish this in this study since this study population is from different parts of the country and the history cannot be verified. Cases of manhandling and cruelty were very prevalent amongst the abattoir workers, while slaughter procedures are inhumane and induced suffering to the cattle. This is similar to incidents previously described elsewhere in a slaughter slabs in Oyo State (Adeyemo et al., 2009). Many cases of poorly conducted ante-mortem inspections (AMI) were observed with implications for risk of transfers to humans of zoonotic and infectious diseases from cattle (FAO, 1994). Since the primary objectives of AMI is previously well described, a poorly conducted AMI as observed in the Bodija Municipal

Abattoir exposes the consuming public and the abattoir workers to tremendous health hazards. In addition, the quality of water used at the abattoir have been known to be heavily contaminated with faecal and other pathogenic microorganism from previous study (Adeyemo et al., 2009) and the quantity used per animal was grossly insufficient, thus the risk of human enteric infections and food-borne diseases associated with meat consumption from this abattoir is possible. Previous worker had recommended a volume of approximately 650 L of water per cow in the Nigerian abattoir (Alonge, 2001). The abattoir workers, including the touts, slaughter staff, blood meal producers, veterinary workers and other visitors are similarly at the risk of such infection described earlier as well as zoonotic diseases since they operate without any protective clothing. In view of the aforementioned, it becomes mandatory to enforce some level of protective material for such workers within the abattoir operations. Furthermore, it was found out that the abattoir floor was heavily contaminated with intestinal contents of the slaughtered cattle and subsequent carcasses were processed on the same floor, thus aiding cross contamination. Standard practice elsewhere and more hygienic methods of carcass processing suggests that abattoir slaughter should be carried out with the animals hoisted on rails and moved from one section to another without touching the ground (FAO, 1991).

A sero-prevalence of 5.45% was observed amongst the slaughtered cattle. This low sero-prevalence can be attributed to the likelihood that the sensitivity of the test falls below the threshold to determine the actual prevalence level of this disease in the surveyed population. This is because RBPT had previously been confirmed to have a low sensitivity (66.7%), although it is highly specific (98.9%) (Fosgate et al., 2002). Whether there is some degree of overestimation due to possible vaccination cannot be established in this study as information concerning vaccination status was not available. In addition, Gall and Nielsen (2004) had similarly confirmed that some serological test have higher performance indices than RBPT. It should be stated that the nature of the transhumant management of cattle in Nigeria is very conducive for the spread of an infectious agent like *B. abortus*. Livestock raised in the north part of Nigeria are moved down south for purposes of trade and feed resources, especially in the drier period of the year, and are returned up north following the end of the drought or dry period (Ducrottoy et al., 2014). Also young and susceptible animals are likely to become infected by carriers and sick animals at communal grazing and watering sites on the transhumance route and possibly returned up north with new infections. Such animals may only be presented at the second trade season to the south as adult, with full or partial manifestations of brucellosis. In addition, about 2.5 to 9% of heifers born from seropositive cows may be latently infected but serologically negative until when such heifers are

Table 1. Cattle slaughter figure and body conditions of sampled cattle over a 10-week study period.

Weeks of study	No. slaughtered	Bulls	Cows	Number sampled	Body conditions		
					Good	Fair	Poor
Week 1	1721	502	1219	22	8	9	6
Week 2	1609	693	916	18	8	6	4
Week 3	1622	487	1135	20	8	9	3
Week 4	1582	798	784	20	10	4	6
Week 5	1520	422	1098	20	6	13	1
Week 6	1542	539	1003	20	12	7	1
Week 7	1609	622	987	20	7	11	2
Week 8	1425	698	727	20	6	7	7
Week 9	1612	616	996	20	7	7	6
Week 10	1980	691	1289	20	6	12	2
Total	17912	6596	11316	220	87	92	41

Table 2. Breed, sex and age distributions of cattle sampled.

Breed	Sex of cattle		Age		Total (%)
	Male	Female	<1 year	> 1 year	
White Fulani	38	50	7	81	88 (40.0)
Red Bororo	31	37	2	66	68 (30.9)
Sokoto Gudali	17	13	0	30	30 (13.6)
Adamawa Gudali	3	2	0	5	5 (2.3)
Keteku	8	5	0	13	13 (5.9)
Kuri	5	3	0	8	8 (3.6)
Muturu	6	2	0	8	8 (3.6)
Total	108 (49.1%)	112 (50.9%)	9 (4.1%)	211 (95.9%)	220

Table 3. Distribution of RBPT positive samples with breed, gender, age and physical Conditions.

Breed	No. +Ve	% +Ve	Range at CI _{95%}	% +Ve within breed	Sex		Age		Physical condition			Total No. of cattle tested
					+Ve Bull	+Ve Cow	+Ve Calve	+Ve Adult	+Ve Good	+Ve Fair	+Ve Poor	
White Fulani	6	2.73	1.26-5.82	6.81	1	5	0	6	1	2	3	88
Red Bororo	2	0.91	0.25-3.25	2.94	1	1	0	2	0	1	1	68
Sokoto Gudali	2	0.91	0.25-3.25	6.67	2	0	0	2	2	0	0	30
Adamawa Gudali	0	0.00	-	0.00	0	0	0	0	0	0	0	5
Keteku	1	0.45	0.08-2.53	12.50	0	1	0	1	0	1	0	13
Kuri	1	0.45	0.08-2.53	12.50	1	0	0	1	0	1	0	8
Muturu	0	0.00	-	0.00	0	0	0	0	0	0	0	8
Total	12	5.45	2.99-9.09	-	5	7	0	12	3	5	4	220

Scoring of animal body conditions were as described by Marston, 2005. Good: Moderate to fat; Fair: Borderline to moderate; Poor: Thin to borderline.

pregnant for the first time or later (Bishop et al., 1994), and this could be the principal reason why all the young stock in this study samples tested negative. Other workers have also confirmed that significant increase in brucellosis sero-positivity increases with the age of sampled animals (Ducrottoy et al., 2014).

The typical Nigerian stock for purposes of milk production

is the White Fulani (Bunaji) breed, and 50% of all seropositive animals in this study were of this breed. Traditionally, wives of herdsmen prepare such milk (which may not be sufficiently pasteurized to kill the resident micro-organisms) and its products (soft cheeses) for sale to the consuming public (Cadmus et al., 2010). In addition, since close associations are known to exist

between the traditional stockmen and their stock, these stockmen are likely to be more predisposed to cases of brucellosis. Both of these situations predispose human to the risk of zoonotic transfer and the perpetuation of the infection in a herd that can easily be passed on prior to and during slaughter.

In conclusion, although calfhood vaccination, regular surveillance for early detection of brucella, and test and slaughter policies may be a standard in developed economies and conducted regularly, it is not routinely implemented in Nigeria, primarily for economic reasons, low prioritization of the disease against other animal and human diseases by government and lack of adequate veterinary infrastructure (McDermott et al., 2013). Since cattle are not routinely tested for brucellosis in Nigeria, precautionary measures against human infection particularly in the transhumant herds becomes vital. Consequently, slaughter processes at the abattoir pose severe zoonotic risk, especially to the abattoir workers where humans are often in close contact with blood and aerosols during the slaughter process. Health education on the importance and risk of zoonotic potentials of work-associated infection with brucellosis becomes necessary for the slaughter-men, butchers, butcher's assistants (slaughter hands), animal health workers, veterinarians and blood meal processors. The use of protective clothing and observance of high level of hygiene in the course of their work will reduce such risks. The early recognition of symptoms of human brucellosis (undulant fever, hygroma, weakness, muscle-aches and joint pains) will assist in mitigating infection and control of the disease. It should be appropriate for government to legislate and implement rapid penside test at the lairage for *B. abortus* to reduce the zoonotic risks associated with slaughtering of positive cattle.

Conflict of interests

The authors declare that they have no conflict of interest.

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Full Length Research Paper

Haemato-biochemical parameters as prognostic indicators in elephant colic

Boon Allwin^{1*}, P. A. Kalaignan² and N. R. Senthil³

¹Department of Wildlife Science, Madras Veterinary College, Chennai, India.

²Bannerghatta Biological Park, Bangalore, India.

³Central Clinical Laboratory, Madras Veterinary College, Chennai, India.

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Haemato-biochemical parameters were found to be useful prognostic indicators in assessing the severity of colic. Significant increase in packed cell volume (PCV), leucocytosis, neutrophilia and lymphocytosis was observed in case of severe colicky elephants. Significant increase in the blood urea nitrogen (BUN) and creatinine levels was observed in moderate and severe colic cases. In severely colicky elephants, the elevation of glucose was found to be double than that of mild and moderate colicky elephants. Hypoproteinemia and hypoalbuminemia was observed in all the three groups of colicky elephants. Lactate dehydrogenase (LDH) was found to be significantly elevated in elephants with moderate and severe colic cases.

Key words: Elephant colic, haematology-Hb, packed cell volume (PCV), total erythrocyte count (TEC), white blood cell (WBC) and DC-biochemistry-blood urea nitrogen (BUN), creatinine, glucose, total protein and albumin.

INTRODUCTION

Colic is considered as the most frequent emergency encountered in elephant practices worldwide. Although a correct clinical diagnosis and localization of the site and type of the intestinal lesion is often difficult, it is necessary for diagnosis and prognosis (Blikslager and Roberts, 1995). The prognosis in these cases therefore remains the deciding factor for whether to treat or not and the type of treatment. The prognostication for elephant colic is basically categorized into prognosis for life, prognosis for future use and prognosis for a future free of colic. In recent times, studies were performed to identify clinical and laboratory variables that could be used to predict survival chances of the affected elephants (Moore,

2006). In this backdrop, the present study is planned to evaluate the usefulness of commonly assessed clinical and laboratory parameters for prognostication in elephants with colic (Turner et al., 1984). Currently, no major studies in this regard are available in this part of the country. The objective of the present study was to evaluate the haemato-biochemical parameters as prognostic indicators in elephant colic.

MATERIALS AND METHODS

Thirty clinical cases of colic in India involving elephants, aged between 2 and 60 years old in the various elephant camps in Tamil

*Corresponding author. E-mail: boonallwin@gmail.com.

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Nadu, Karnataka and elephants in the annual rejuvenation camp were used for the clinical study. Ten apparently healthy elephants were used as healthy control. The study composed of four groups as follows: Group I healthy elephants (n=10), Group II mild colic (n=13), Group III moderate colic (n=9) and Group IV severe colic (n=8).

Blood samples were collected from each animal from auricular vein as per standard protocols of Youssef et al. (2009). A total of 5 ml of blood was collected from auricular vein; 2 ml of blood was transferred into a vacutainer containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant for the hematological studies and 3 ml of blood was transferred into a vacutainer without anti-coagulant for serum collection.

Hematological analysis was done using an automated hematology analyzer (MINDARY-BC-2800 VET) and hematological parameters such as hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), and platelet count were assessed as per standard protocols of Piccione et al. (2005). Serum samples were subjected for estimation of blood urea nitrogen (BUN), creatinine, total serum protein, glucose, albumin, globulin and lactate dehydrogenase using automated biochemical analyzer (A – 15 BIO SYSTEM). Statistical analysis was carried out using statistical software package SPSS – 12.0. The results were presented in figures, tables and discussed critically.

RESULTS

The mean \pm standard error (SE) values for hemogram (Hb, PCV, TEC, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of control and different groups of colic cases in elephants are shown in Table 1.

No significant differences were observed in the mean of hemoglobin, TEC and MCH among different groups. The PCV and MCV values in severe colic cases showed a significant increase as compared to the control, mild and moderate colic cases. No significant difference was observed in PCV and MCV values between mild and moderate type of colic cases. There was no significant difference of MCHC value within the colic groups, whereas significant difference was observed in these three groups when compared with control group elephants.

The mean \pm SE values of total and differential leukocyte count are shown in Table 2. The total and differential leukocyte count was highly significant in severe colic cases when compared with the control, mild and moderate group elephants. The absolute neutrophil count was highly significant in severe colic, when compared with control, mild and moderate group of elephants. There was no significant difference in absolute lymphocyte count between mild and moderate colic cases, whereas significant increase was observed when compared with control group in severe colic cases compared with mild and moderate colic cases.

The mean \pm SE values of BUN, creatinine, blood glucose, total protein, albumin and lactate dehydrogenase (LDH) of control, mild, moderate and

severe colic groups are shown in Table 3. No significant differences in the mean value of blood urea nitrogen and creatinine were observed among control and mild colic cases. There was highly significant increase in the BUN level in moderate and severe colic when compared with control and mild type of colic, and being higher in Group IV than in Group III. Significant increase in the creatinine level in moderate and severe colic cases was noticed as compared to the control group and mild colic group elephants.

In the present study, there was a highly significant increase in glucose level in severe colic cases as compared to the control, mild and moderate groups. The increase in glucose levels may be due to stress or activation of catecholamines leading to glucogenolysis. No significant difference was observed in glucose level between control, mild and moderate colic cases. There was a significant decrease in total protein and serum albumin in different colicky elephants when compared with the control group, with greater difference in the most severe colics.

In the present study, there was a highly significant increase of LDH value in different colic groups. A significant difference in LDH value was noticed between moderate and severe colic cases and these two groups in relation to Groups I and II. There was no significant difference in LDH value between control and mild colic cases. Significantly increased value of LDH was observed in severe colic elephants as compared to the control, mild and moderate groups.

DISCUSSION

In the present study, 80% of the severe colicky elephants had died and their hematocrit was found to be the highest (59.35 ± 0.73) among all the groups. This proved that PCV is one of the best prognostic parameter in colicky elephants. These findings were in accordance with Parry et al. (1983), who reported that PCV values of 30, 45, 60 and 65% were associated with probable survival rates of 93, 64, 20 and 10%, respectively.

In the present study, significant leucocytosis, neutrophilia and lymphocytosis was observed in the case of severe colicky elephants. Thus, white blood cell (WBC) count and differential count also proved to be an important prognostic variable in assessing the severity of colic. The findings observed in this study were in accordance with Sabev and Kannakov (2008) who reported that there was marked leukocytosis (left shift) with neutrophilia in colic cases.

In the present study, in severe colicky elephants, azotemia was almost double that of mild and moderate colicky elephants which emphasized the utility of BUN and creatinine as important biochemical parameters for prognostication (White, 1990). These findings were agreement with Southwood (2006) who reported that it

Table 1. Mean \pm SE value of erythrogram for control and different groups of colicky elephants.

Parameter	Group I (Control) n=10	Group II (Mild) n=13 Survival 100%	Group III (Moderate) n=9 survival 100%	Group IV (Severe) n=8 non survival 20%	F value
Hemoglobin (g/dl)	17.38 \pm 0.58 ^a	17.06 \pm 0.62 ^a	16.95 \pm 0.39 ^a	17.86 \pm 0.43 ^a	0.634 ^{NS}
PCV (%)	45.48 \pm 1.33 ^a	51.02 \pm 0.28 ^b	53.15 \pm 1.02 ^b	59.35 \pm 0.73 ^c	35.665 ^{**}
TEC ($\times 10^6/\mu\text{l}$)	8.44 \pm 0.11 ^a	8.54 \pm 0.14 ^a	8.67 \pm 0.15 ^a	8.67 \pm 0.14 ^a	0.683 ^{NS}
MCV (fl)	53.92 \pm 1.55 ^a	59.88 \pm 1.02 ^b	61.54 \pm 1.91 ^b	68.62 \pm 1.40 ^c	16.195 ^{**}
MCH (pg)	20.61 \pm 0.70 ^a	19.97 \pm 0.63 ^a	19.59 \pm 0.50 ^a	20.69 \pm 0.75 ^a	0.661 ^{NS}
MCHC (g/dl)	38.57 \pm 1.83 ^a	33.41 \pm 1.13 ^b	31.98 \pm 0.89 ^b	30.14 \pm 0.82 ^b	8.636 ^{**}

Same superscript in row do not differ significantly. NS: Not significant ($P > 0.05$); **Highly significant ($P < 0.01$).

Table 2. Mean \pm SE value of Leukogram for control and different groups of colicky elephants.

Parameter	Group I (Control) n=10	Group II (Mild) [n=13, survival 100%]	Group III (Moderate) [n=9 survival 100%]	Group IV (Severe) [n=8 non survival 20%]	F value
WBC (μl^{-1})	7840 \pm 210.39 ^a	9100 \pm 147.57 ^b	9330 \pm 152.78 ^b	15590 \pm 303.11 ^c	267.067 ^{**}
Absolute neutrophils (μl^{-1})	5116 \pm 133.39 ^a	5906.6 \pm 108.23 ^b	6040.2 \pm 149.84 ^b	11628.4 \pm 283.63 ^c	271.57 ^{**}
Absolute lymphocytes (μl^{-1})	2724 \pm 97.57 ^a	3123 \pm 71.69 ^b	3196.9 \pm 66.42 ^b	3835.2 \pm 200.19 ^c	14.298 ^{**}

Same superscript in row do not differ significantly. NS: Not significant ($P > 0.05$); **Highly significant ($P < 0.01$).

Table 3. Mean \pm SE value of serum biochemistry profile for control and different groups of colicky elephants.

Parameter	Group I (Control) n=10	Group II (Mild) [n=13 survival 100%]	Group III (Moderate) [n=9 survival 100%]	Group IV (Severe) [n=8 non survival 20%]	F value
BUN (mg/dl)	12.13 \pm 0.28 ^a	14.04 \pm 0.21 ^a	17.42 \pm 0.53 ^b	35.86 \pm 1.27 ^c	234.873 ^{**}
Creatinine (mg/dl)	0.75 \pm 0.08 ^a	1.04 \pm 0.09 ^a	1.52 \pm 0.02 ^b	2.57 \pm 0.22 ^c	40.669 ^{**}
Glucose (mg/dl)	75.70 \pm 0.70 ^a	76.10 \pm 0.82 ^a	86.40 \pm 3.52 ^a	168.10 \pm 10.88 ^b	60.478 ^{**}
Total protein (g/dl)	6.24 \pm 0.08 ^a	5.91 \pm 0.06 ^b	5.21 \pm 0.03 ^c	4.63 \pm 0.08 ^d	121.064 ^{**}
Albumin (g/dl)	2.92 \pm 0.02 ^a	2.08 \pm 0.13 ^b	1.75 \pm 0.08 ^c	1.45 \pm 0.07 ^d	32.479 ^{**}
LDH (U/L)	164.70 \pm 2.26 ^a	175.00 \pm 2.55 ^a	222.80 \pm 4.56 ^b	482.20 \pm 17.66 ^c	259.989 ^{**}

Same superscript in row do not differ significantly. NS: Not significant ($P > 0.05$); **Highly significant ($P < 0.01$).

was attributed to decreased renal blood flow resulting from systemic hypotension.

In the present study, in severely colicky elephants, the elevation of glucose was found to be double than that of mild and moderate colicky elephants. In severe colicky elephants 80% of them died and this proved that glucose is also an important parameter in prognosticating the elephant colic. The changes in glucose level and survival observed in the study were in accordance with the previous reports of Parry et al. (1983) who reported that blood glucose values of 90, 200 and 235 mg/dl corresponded to survival probabilities of 65, 46 and 45%, respectively.

In the present study, both total protein and albumin

were significantly reduced in all the three groups of colicky elephants. These findings were in accordance with Southwood (2006) who reported that high haematocrit and low protein was found to be associated with less favorable prognosis for elephants with colic. Hypoproteinemia and hypoalbuminemia was also documented in an elephant with severe colic due to dorsal colitis (Galvin et al., 2004).

In this study, the LDH was found to be significantly elevated in elephants with moderate colic (222.80 \pm 4.56) and severe colic (482.20 \pm 17.66). This underscored the utility of LDH as a prognostic variable in elephants with colic. The findings of LDH elevation in this study was in accordance with the report of Sabev and Kanakev (2008)

who reported extremely elevated LDH activity in elephants with caecal impaction. Haemato biochemical parameters were considered to be very useful for prognostication of elephant colic.

Conflict of interest

Authors have none to declare.

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Review

A review on major bacterial causes of calf diarrhea and its diagnostic method

Yimer Muktar^{1*}, Gezhagne Mamo², Biruk Tesfaye² and Dinaol Belina¹

¹College of Veterinary Medicine, Haramaya University, Ethiopia, P.O.Box 138, Dire Dawa, Ethiopia.

²College of Veterinary Medicine and Agriculture, Addis Ababa University, P.O. Box 34, Debre Zeit, Ethiopia.

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Calf diarrheic diseases result from complex interactions of the environment. Infectious agents and the calf itself are the major constraints for raising replacement stock. Calf diarrhea is a multi factorial disease entity that can have serious financial and animal welfare implications in both dairy and beef sucker herds and is one of the most common diseases reported in calves up to 3 months old. Among the bacterial causes of diarrhea in neonatal food animals, *Escherichia coli* and *Salmonella* species are the most common and economically important ones. *Clostridium perfringens* and *Campylobacter* species have also been identified as causes of enteric diseases in calf diarrhea other, Non-infectious factors, such as insufficient uptake of colostrum, poor sanitation, stress, overcrowding in the calf pens and cold weather, could cause neonatal calf diarrhea. The most prominent virulence factors identified in bacterial diarrhea are expression of fimbrial (pili) antigens that enables the bacteria to adhere and to colonize the luminal surface of the small bowel and elaboration of one or more enterotoxins that influence intestinal secretion of fluids. Various laboratory methods have been applied for the detection of infectious agents of calf diarrhea in fecal sample such as, bacterial culture, electron microscopy, molecular based techniques (PCR, DNA microarray) and serological techniques (enzyme-linked immunosorbent assay, latex agglutination test). Accurate and rapid early confirmation of the etiology in the disease outbreak as well as improving the various management factors are advised, for effective control and prevention of enteric disease in newborn calves. Treatment with rehydration solutions and provision of dry and warm conditions are vital in the treatment of calf diarrhoea.

Key Words: Bacteria, calf diarrhoea, *Escherichia coli*, *Salmonella* species, risk factor.

INTRODUCTION

The future of any dairy production depends, among other things, on the successful raising of calves and heifers for replacement. Under modern dairy production in the developed world, the average length of time a cow stays in a milking herd is about four years and, therefore, 25% of the milking herd must be replaced each year (Bath et al., 2012).

Generally, calf diarrhea result from complex interaction of the environment, infectious agents and the calf itself are the major constraints for raising replacement stock. The impacts of calf diseases could be direct and indirect through increased treatment expenses, decreased lifetime productivity and survivorship (Waltner-Toews et al., 1986a; Randhawa et al., 2012).

*Corresponding author. E-mail: yimermkr21@gmail.com; yimer.muktar@haramaya.edu.et.

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Calf diarrhea is a multifactorial disease entity that can have serious financial and animal welfare implications in both dairy and beef sucker herds. It has been estimated that 75% of early calf mortality in dairy herds is caused by acute diarrhea in the pre-weaning period and also, a commonly reported disease in young animal and still a major cause of productivity and economic loss to cattle producers and also a cause of high morbidity and mortality in the cattle industry worldwide (Uhde et al., 2008; Bartels et al., 2010). Diarrhea is one of the most common diseases reported in calves up to three months old (Svensson et al., 2003). However, calf diarrhea was perceived as a minor problem by dairy producers, while the beef producers did not consider it a problem at all (Roderick and Hovi, 1999).

Various infectious agents such as viruses, bacteria, and protozoa are involved in calf diarrhea (Smith, 2009). Among these agents that have been implicated in calf diarrhea, bovine corona virus (BCoV), bovine rotavirus (BRV) group A, and bovine viral diarrhea virus (BVDV) act as viral agents, *Salmonella* species, *E. coli* K99+ and *Clostridium* species act as bacterial agents and *Cryptosporidium* spp. as a protozoan agent (Bhat et al., 2012; Bhat et al., 2013; Singla et al., 2013). *Salmonella* species and *E. coli* K99+ are known as the most common pathogens identified in scouring calves less than two months of age (Acha et al., 2004).

According to Cho (2012), 80% of diarrheic calves tested were positive for at least one of the target enteric pathogens, suggesting that the infectious factor is still a major cause of calf diarrhea. More than 50% of the diarrheic calves tested were concurrently infected with more than one pathogen. Co-infection with two pathogens were the most common finding (31%) with up to six pathogens detected in 1% of the fecal samples from diarrheic calves. The majority of diarrheic cases were identified among 0 to 4 week old calves and concentrated among calves at 0 to 2 weeks of age. High frequency of co-infection by multiple pathogens in young animals emphasizes that interventions for calf diarrhea should be focused on husbandry and management strategies, including assurance of colostrum intake, hygiene, reduction of population density, or modified components of the sand hills calving system (Larson and Tyler, 2005). Many of these enteropathogens cause severe intestinal lesions, alterations in enzyme activity, and alterations in nutrient transport mechanisms, or a combination of these effects. Infectious diarrhea of neonatal animals is one of the most common and economically devastating conditions encountered in the animal agriculture industry (Wudu, 2008).

Non-infectious factors, such as insufficient uptake of colostrum, poor sanitation, stress and cold weather could cause neonatal calf diarrhea. Due to its poor immune capability, a newborn calf is vulnerable to infection. The main risk factors increasing the exposure to infection and further lowering the defense mechanism within the calf in

early life are: poor hygiene and overcrowding in the calving facility, high relative humidity, low temperature of the incoming air, contamination of the incoming air inadequate ventilation, close proximity to adult cows, mixing of different age groups and poor stockman ship or motivation of the herds person responsible for the calves (Lance et al., 1992).

Various laboratory methods have been applied for the detection of infectious agents in feces. Historically, virus isolation, electron microscopy, enzyme-linked immunosorbent assay, latex agglutination test, bacterial culture, direct microscopy of fecal smear and/or fecal flotation have been commonly used to test fecal samples for enteric pathogens (Fotedar, 200; Meir et al., 2010).

Acute infectious diarrhea encountered in a herd is often difficult to manage because of the large number of potential enteropathogens involved, differences in individual animal immunity within the herd, population dynamics, environmental stresses, nutritional status—and difficulty in establishing an etiologic diagnosis (Waltner-Toews et al., 1986b). The etiologic diagnosis is not determined for a large percentage of cases of neonatal diarrheas. However, from infectious bacterial enteropathogens, *E. coli*, *S. species* and *Clostridium* species are the important bacterial causes of calf diarrhea which can be diagnosed and confirmed using the available laboratory techniques. Accurate and rapid confirmation of the etiology early in the disease outbreak can aid in quick implementation of appropriate interventions or prevention measures in the herd to decrease economic losses (McGuirk, 2008; Meir et al., 2010). Therefore, the objectives of this review is to highlight the major bacterial causes of calf diarrhea and its diagnostic method in dairy calves, and assess the zoonotic importance of bacteria involved in calf diarrhea.

GENERAL DESCRIPTION OF BACTERIA INVOLVED IN CALF DIARRHEA

Farm animals are born into environments with many potential enteropathogens and are initially exposed to resident micro flora in the vagina of the dam and subsequently to microbes harbored by herd mates. Some microorganisms are potentially harmful, while others are necessary for normal development and function of the gastrointestinal tract. However, once exposed, the intestinal tract is susceptible to infection with potential enteropathogens, and in the absence of protective antibodies, various enteropathogens can become established and cause enteric disease.

Most cases of calf diarrhea are likely to be mixed infections, where more than one of the pathogenic agents is present. The major bacterial infectious agents that have been implicated in calf diarrhea are *S. species*, *E. coli* K99+, and *Clostridium* spp. mixed infections with rotavirus and cryptosporidium appear to be the most

common. Many cases of scour proceed very rapidly, causing severe dehydration and metabolic imbalance within a few hours of the onset of disease. *E. coli* (K99 and F41) can cause very severe scour and dehydration in calves of less than one week old. Diarrhea caused by *E. coli* can occur as early as 24 h after birth, but seldom occurs after three days of age unless it occurs as part of a mixed infection with rotavirus and cryptosporidium species. *E. coli* can also invade the bloodstream and cause colisepticaemia (Quinn et al., 1994).

Experimental work also suggests that infection with one agent makes the calf more vulnerable to other pathogens. Many surveys indicate that rotavirus is the most common single cause of calf diarrhea in all types of cattle herds, but particularly in dairy and single sucker herds. Cryptosporidium is the second most common pathogen causing calf diarrhea, and slightly more common in beef sucker units than in dairy herds. Coronavirus is the third most common diarrhea agent. Salmonella is isolated often from disease outbreaks, particularly *Salmonella Dublin* in calf rearing units where calves are bought in from multiple sources (Hall et al., 1988; Fagan et al., 1995).

The genus *Escherichia*

Escherichia coli commonly abbreviated as *E. coli*; is a gram negative rod-shaped motile or nonmotile, facultative anaerobic, non-spore forming member of the Enterobacteriaceae family found in the gastrointestinal tract of warm-blooded animals and humans (Frydendahl, 2002). *E. coli* was discovered by German pediatrician and bacteriologist Theodor Escherich in 1885 (Feng et al., 2002) and is now classified as part of the enterobacteriaceae family of gamma-proteobacteria. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2 (Bentley and Meganathan, 1982) and by preventing the establishment of pathogenic bacteria within the intestine (Hudault et al., 2001; Reid et al., 2001). New strains of *E. coli* evolve through the natural biological process of mutation and through horizontal gene transfer (Lawrence and Ochman, 1998). *E. coli* is a facultative habitant of the gastrointestinal tract, and also found in the environment. However, the infection is present due to break of the protection barrier, extreme pathogenic bacteria type or immunosuppression. Clinical disease due to *E. coli* in calves may be present as enteric or septicemic illness, being one of the most important causes of neonatal mortality in dairy calves. (Lofstedt et al., 1999). Some strains develop traits can be harmful to a host animal. Diarrhea in calves is commonly caused by enterotoxigenic *E. coli* (ETEC) more recently; attaching and effacing *E. coli* (AEEC) and Shiga toxin-producing *E. coli* (STEC) have also been identified as causes of diarrhea and dysentery in calves (Mainil et al., 1993).

More virulent strains, such as *E. coli* O157: H7 cause serious illness or death in the elderly, the very young or the immunocompromised (Hudault et al., 2001). Identification of certain virulence factors has assisted in defining mechanisms by which various strains result to different diarrheal syndromes, commensal *E. coli* strains rarely contain virulence genes (Boerlin et al., 2005).

E. coli can be classified into six pathogroups based on their virulence scheme: ETEC; shiga toxin-producing *E. coli*; enteropathogenic *E. coli* (EPEC); enteroinvasive *E. coli* (EAEC); enteroaggressive *E. coli*; and enterohaemorrhagic *E. coli* (EHEC) (Kaper et al., 2004). ETEC, EPEC, and EHEC are the diarrheagenic types described as occurring in young farm animals. The importance of ETEC in the etiology of diarrhea among calves, lambs and pigs is well recognized and these organisms should not be confused with the rare EPEC and EHEC types that cause the less-common diarrheal syndromes. Among these pathogroups, the most common cause of neonatal diarrhea is ETEC stains that are producing the K99 (F5) adhesion antigen and also the heat-stable enterotoxin (Nataro and Kaper, 1998). Neonatal calves are most susceptible to ETEC infection during first 4 days after birth and develop "watery" diarrhea if infected (Foster and Smith, 2009). Following ingestion, ETEC infects the gut epithelium and multiplies in enterocytes in intestinal villi. The distal portion of small intestine is the most favorable environment of ETEC colonization due to the low pH (less than 6.5). The bacteria express the K99 antigen for the attachment (Francis et al., 1989). As colonized on the gut epithelium, heat stable toxin is induced by ETEC and causes the secretory diarrhea.

The genus *Salmonella*

Salmonella species are facultative anaerobic gram-negative rods within the family of enterobacteriaceae, they can survive and multiply in the environment as a result of fecal shedding. There are approximately 2,500 known serovars in the *Salmonella* genus (Davies, 2008). The most common serotype isolated is *Salmonella typhimurium*. *S. typhimurium* DT104 exhibits multiple resistances to the commonly used antibiotics (Jones et al., 2002). *S. enterica* colonizes the gastrointestinal tract of a wide range of hosts (Wray, 2000). *S. enterica* serovar *typhimurium* and *S. dublin* are the most common etiology of salmonellosis in cattle (Sojka et al., 1977). *Salmonella* infection has a wide range of clinical manifestation from asymptomatic to clinical salmonellosis. Acute diarrheal disease is most common with *S. typhimurium* and systemic disease with *S. dublin* in cattle. Infected cattle can serve as source of zoonosis through food-borne or direct contact routes (Mead et al., 1999). *S. dublin*, host specific, and *S. typhimurium*, not host-specific, are quoted to be the most common serovar

in bovines (Venter et al., 1994), both affecting calves severely between six and twelve weeks of age and in the first three weeks of age, respectively (Bisping and Amtsberg, 1988). Due to management practices, the disease is more commonly found in dairy than in beef cattle (Venter et al., 1994). Source for infection are mainly latent carriers or contaminated environment, where *Salmonella can* persist for a long period. The infective dose, predisposing factors and the immunity status of the hosts determine the outcome of the infection (Venter et al., 1994). The peracute form is often fatal with signs of diarrhea and septicemia. The acute form goes along with fever, inappetence, diarrhea and polypnoea. In chronic cases of *salmonellosis*, calves are unthrifty, have long scruffy hair and are stunted (Venter et al., 1994).

The genus *Campylobacter*

The first description of a bacterium belonging to the genus *Campylobacter* is attributed to Theodore Escherich at the end of the 19th century. *Campylobacter* has long been recognized as a pathogen and commensal organism of animals and it is one of the most common causes of bacterial gastroenteritis in humans worldwide (Allos, 2001). It is adapted to the intestinal tract of warm-blooded animals and does not normally replicate outside this environmental niche and is widely distributed among animals. *Campylobacter jejuni* is the most common cause of bacterial gastrointestinal disease in many western industrialized countries (Tauxe, 1992). In contrast to most other animals, in which *Campylobacter* does not cause any symptoms, *Campylobacter* species, especially *C. jejuni*, may cause diarrhea in calves (Diker et al., 1990; Schulze, 1992). In addition to the potential risk of *Campylobacter* in contributing to enteritis in calves, calves might act as a reservoir for *Campylobacter* spp. and can be a source of human infection, either by direct contact or through fecal contamination of food and water.

Campylobacter species often inhabit the bovine intestinal tract, particularly of calves. The overall prevalence of *C. jejuni* in calves during the first 3 months of life on large calf farms was 39% in a Swiss (Busato et al., 1999). In a Danish study, (Nielsen, 2002) 20 out of 24 cattle herds were infected, and young animals had a higher prevalence than older animals. In 40% of infected herds, all *C. jejuni* isolates had the identical serotype and pulsed-field gel electrophoresis type. Prevalence of campylobacter infection in a multiple-herd study of adult beef cattle was 5% in California (Hoar et al., 2001).

The genus *Clostridium* Clostridia species are gram-positive bacteria, obligate anaerobes capable of producing endospores. Individual cells are rod-shaped or spindle, and consist of around 100 species that include common free-living bacteria and most importantly pathogens (Lewis, 2011). Clostridia species are

responsible for a wide range of diseases in mammals and birds (Van Immerseel et al., 2004) and can be found in the intestinal tract of human, animals, insects and in soil. *Clostridium* organisms are normal flora of cattle and only become problematic with dietary stress, injury, changes in management, parasitism that results in production of potent toxins (Popoff and Bouvet, 2009). Clostridia species produce the highest number of toxins of any type of bacteria and are involved in severe diseases in animals. Most of the clostridial toxins are responsible for gangrenes and gastrointestinal diseases. Presence of the organism in the intestine is not sufficient to cause diseases. Clostridial toxins are classified into 5 toxinotypes (A, B, C, D and E) according to the production of 4 major toxins, namely alpha (CPA), beta (CPB), epsilon (ETX) and iota (Rood, 1998). *C. perfringens* can produce up to 15 toxins including lethal toxins as perfringolysin O (PFO), enterotoxin (CPE) and beta2 toxin (CPB2) (Gkiourtzidis et al., 2001) summarized in Table 1.

Some type A strains produce an enterotoxin that causes diarrhoea in humans and most likely also in various domestic animals. *C. perfringens* type A strains have been associated with intestinal disorders in horses, piglets, dogs and calves. Thus, the detection of *C. perfringens* toxin types and subtypes is critical for a better understanding of the epidemiology of *C. perfringens* infections and may be helpful in the development of effective preventive measures (Baums et al., 2004).

Clostridium difficile has been implicated as an important etiological agent in antimicrobial associated diarrheal disease and pseudomembranous colitis (Bartlett et al., 1980). It is a recognized pathogen in neonatal pigs and may contribute to enteritis in calves while recent evidence suggests that the epidemiology of *C. difficile* associated disease (CDAD) is increasing in incidence and severity (Boerlin et al., 2005). These changes are due, at least in part, to the emergence of a more virulent *C. difficile* strain, designated NAP1 (based on its pulsed-field gel electrophoresis (PFGE) pattern, by restriction endonuclease analysis (REA) and toxinotype III by polymerase chain reaction (PCR) characterization of the pathogenicity locus by PCR ribotyping).

PATHOGENESIS AND VIRULENCE FACTORS

Escherichia coli

Investigations into *E. coli* strains associated with individual cases or outbreaks of diarrhea among neonatal calves, lambs, pigs, and humans have helped to determine specific virulence factors that can be used to distinguish between pathogenic and commensal strains (Okerman, 1987). Virulence factors in *E. coli* include the ability to resist phagocytosis, utilization of highly efficient iron acquisition systems, resistance to killing by serum,

Table 1. Major lethal toxins of *C. perfringens* for type determination.

Type	Toxins			
	Alpha	Beta	Epsilon	Iota
A	++	-	-	-
B	+	++	+	-
C	+	++		-
D	+	-	++	-
E	+	-	-	++

Source: (Niilo, 1980). ++ = Produced as a predominant toxic fraction, + = Produced in smaller quantities, - = Not produced.

production of colicins and adhere, colonize, and invade the hosts' cells. Further to these are the secretion systems, production of cell surface molecules, transport and siderophore formation. Mortality survey confirmed scour as the main clinical sign (48%) in camel calves, which were born alive but died within the first month of life (Hamouda et al., 2010). The pathogenicity of STEC O157:H7 is associated with a number of virulence factors, including Shiga toxin 1 (encoded by the *stx1* gene), Shiga toxin 2 (encoded by the *stx2* gene), intimin (encoded by the *eae A* gene) and enterohaemolysin (encoded by the *Ehly* gene) (Kang et al., 2004).

Two of the more prominent virulence factors identified for ETEC strains are expression of fimbrial (pili) antigens that enables the bacteria to adhere to and to colonize the luminal surface of the small bowel and elaboration of one or more enterotoxins that influence intestinal secretion of fluids (Holland, 1990) through increased cellular concentrations of cyclic AMP (cAMP) or cGMP. Although, the association with serotypes or serogroups does not confer virulence, results of several studies have shown that ETEC strains are limited to a few serotypes or serogroups (Soderlind et al., 1988). The most common observed fimbriae on ETEC from calves with diarrhoea are F5, also named K99 and F41, but strains with F165 fimbriae have also been isolated (Contrepolis et al., 1989). K99 antigen is a fimbrial adhesion distinct from the capsular polysaccharide K antigens (Orskov et al., 1975). Two biological classes of enterotoxins are produced by ETEC: heat labile (LT) and heat stable (STa and STb) (Scotland et al., 1985), and most bovine ETEC produce STa enterotoxin and K99 fimbriae (Kaeckenbeeck, 1981).

Salmonella

In cattle, enteric salmonellosis is very common. Various stress factors influence the outcome of the infection (Fenwick and Collett, 2004). Initial infection may be followed by bacteremia and dissemination to several organs. In pregnant animals, abortion may occur. Resistant *Salmonella* infections of calves are very common, so disease caused by *Salmonella* is often very

severe (Radostits et al., 2007). Animals that recover from *Salmonella* infections may become carriers for life, shedding organisms sporadically in their faeces (Radostits et al., 2007). The basic virulence mechanism of *Salmonella* includes the ability to invade the intestinal mucosa, to multiply in lymphoid tissues and to evade host defense systems, leading to systemic disease. For the pathogenesis of *Salmonella*, the organism should be capable of invading intestinal epithelial cells, surviving within macrophages and causing enteropathogenicity (Tsolis et al., 1999; Holt, 2000). *Salmonella* colonizes in M-cell, enterocytes and tonsillar tissue (Reis et al., 2003). In lymphoid tissue infection, *Salmonella* easily spreads throughout the whole body by invading mononuclear cell and phagocytes (Holt, 2000). *Salmonella* pathogenicity island 1 (SPI-1) and SPI-5 are known to be involved in the type III secretion system, and are mainly responsible for *Salmonella* induced diarrhea in calves (Tsolis et al., 1999). SPI-2 is involved in the second type III secretion system and is responsible for intracellular survival of the organism (Ochman et al., 1996).

Clostridium

Organisms are normal flora of cattle and only become problematic with dietary stress, injury, changes in management, and parasitism result in production of potent toxins. *Clostridium perfringens* are part of the normal intestinal flora. However, it causes enterotoxemia syndromes of cows and calves. The role of some of these toxins in the pathogenicity of disease has not been clarified yet. *C. perfringens* strains harboring *cpb2* have frequently been associated with enterotoxaemia in sheep and goat (Uzal and Songer, 2008).

The virulence of *C. perfringens* is determined by its prolific toxin-producing ability, including enterotoxins. *C. perfringens* strains are divided into five toxin types (A, B, C, D and E) on the basis of the production of four major lethal toxins: α , β , ϵ and ι (Al-Khalidi et al., 2004). Type A strains produce alpha (α) toxin only; type B strains produce α , beta (β) and epsilon (ϵ) toxins; type C type strains produce α and β toxins; type D strains produce α

toxins and type E strains produce α and ι (iota) toxins. Among these types, type C has been frequently reported in conjunction with calf diarrhea (Rings, 2004) but not as frequently as some other enteric pathogens such as *E. coli* and *Salmonella*. The toxin is the main lethal toxin, and functions in cell lysis through hydrolysis of membrane phospholipids. The β toxin is highly trypsin-sensitive and induces mucosal necrosis. The toxin causes lethal enterotoxemia in domestic animal, and the ι toxin is responsible for dermo necrosis due to its high vascular permeability (Songer, 1997., Petit et al., 1999). Enterotoxin causes diarrhea and intestinal cramping due to its act on epithelial tight junction protein Beta-2 toxin, which is produced from all types of *C. perfringens*, has been recently reported and postulated to have a synergetic function with enterotoxin (Gurjar et al., 2008).

Most domestic animals are susceptible to all types of *C. perfringens* due to the ubiquitous nature of the bacterium in the environment. Newborn calves which have a low level of proteolytic enzymes (example, trypsin) in gastrointestinal track can be easily infected by *C. perfringens* type C as β toxin is recognized as the main virulence factor responsible for clinical signs seen in affected animals. Intestinal lesions in such affected animals are characterized by diffuse or multifocal hemorrhagic necrotizing enteritis and bloody fluid distension (Barker et al., 1993). The pathogenicity of *C. difficile* is due to its ability to produce an extracellular protein that has cytotoxic effects on cells in tissue culture (Donta et al., 1980), and can produce disease in experimental animals similar to that seen in humans.

Campylobacter

Campylobacter jejuni is one of the most important causes of food-borne illness in industrialized nations and diarrhea in children in developing countries (Lylerly et al., 1988). Despite its importance as a pathogen, its virulence mechanisms are just beginning to be understood. The ability of *C. jejuni* to enter non phagocytic cells is thought to be very important for its pathogenesis (Mcsweegan and Walker, 1986). Proteinaceous toxins are relevant in the context of enteropathogenicity which can be classified into two classes depending on their primary mode of action: enterotoxins and cytotoxins. Enterotoxins are secreted proteins with a capacity to bind to a cellular receptor, enter the cell, and elevate intracellular cyclicAMP (cAMP) levels. The prototypes of enterotoxin cytotoxic are vibrio cholerae toxin (CT) and the closely related *E. coli* heat-labile toxin (LT) (Spangler, 1992). Cytotoxins are proteins that kill target cells cytotoxins which act as intracellular or form pores in the cells.

Cytotoxins with intracellular activity generally bind to the cells and are processed before they reach the cell cytoplasm. Different mechanisms of toxicity exist, of which two predominate: inhibition of cellular protein synthesis and inhibition of actin filament formation

examples, of the first type of cytotoxin are shigella dysenteriae toxin and the related *E. coli* Shiga-like toxin (Stx) also known as verotoxin. The two toxins are closely related and contain two subunits, the A subunit with enzymatic activity and a pentamer of B subunits (Olsnes et al., 1981).

Diagnosis

An etiologic diagnosis is useful in selecting specific diagnostic and preventative regimens for bacterial infections. Establishing an etiologic diagnosis for bacterial infections may be more important now that there is some indication that effective vaccines are being developed. Diagnosis of salmonellosis and higatoxigenic *E. coli*, can have public health implications. Once an agent has been identified, one of the major problems is in interpretation whether or not it is responsible for diarrhea in the individual or herd, because most agents can also be found in healthy calves. Identification of one agent in pathologic material does not preclude the possibility that other agents are also contributing to the condition (Naylor, 2002).

Culture techniques are designed to promote the growth and identify bacteria, while restricting the growth of the other bacteria in the sample. Often, these techniques are designed for specific specimens; for example, a sputum sample will be treated to identify organisms that cause pneumonia, while fecal specimens are cultured on selective media to identify organisms that cause diarrhea, while preventing growth of non-pathogenic bacteria. Specimens that are normally sterile, such as blood, urine or spinal fluid, are cultured under conditions designed to grow all possible organisms (Thomson and Bertram, 2001). Once a pathogenic organism has been isolated, it can be further characterized by its morphology, growth patterns such as aerobic or anaerobic growth, patterns of hemolysis and staining. Many scours, regardless of cause, show similar clinical picture.

However, the severity and character of the scour and the age of the affected calves can all help to make a professional judgment for the cause. Frequently, examination of faces samples from a group of calves can identify the organisms present in an outbreak. However, routine tests fail to identify any specific organism and further examinations are required to make an accurate diagnosis, for example, history of predisposing causes, isolation of *E. coli*, postmortem examination (Quinn et al., 2002) and the use of PCR to test for enterotoxigenic genes (Ahmed et al., 2007).

Clinical finding

Disease produced by *E. coli* organism is associated with the feeding of inadequate levels of colostrum. Severe infection results in severe dullness, listlessness and

collapse in calves of less than one week old and is often fatal despite therapy. *E. coli* K99+ causes a watery diarrhea, dehydration, and weakness in 1- to 4-day-old newborn calves. The fimbrial adhesion F5 (K99) promotes the attachment of bacterial cells to glycoproteins on the surface of epithelial cells of the jejunum or ileum and bacterial enterotoxin also causes damage to the epithelial cells, resulting in fluid secretion and diarrhea (Acres, 1985).

Salmonellosis is a bacterial disease of humans and animals. The most common serovar infecting cattle is *S. dublin* and the second most common is *S. typhimurium* (Defra, 2005). The condition has different manifestations in infected animals. An acute generalized infection is seldom seen, but causes a severe condition that can be fatal without effective treatment in vulnerable individuals. An acute intestinal infection causes diarrhea, particularly in young animals. In adults, *S. typhimurium* infection is most commonly associated with diarrhea and dullness (Veling et al., 2002). Calves with *Salmonellosis* may become septicemic and die, or may suffer from necrosis of extremities as a sequel, especially the feet, tail and ear tips. A chronic intestinal infection can be asymptomatic clinically, and often leads to the presence of carrier animals that are not identified as *Salmonella* infected.

The diarrhea caused by *Salmonella* infection is characterized by watery and mucoid diarrhea with the presence of fibrin and blood. Even though *Salmonella* can cause diarrhea in both adult cattle and calves, infection is more common and often causes severe symptoms in 10 day to 3 month old calves (Fossler et al., 2005). *C. jejuni* is the most common cause of bacterial gastrointestinal disease in many western industrialized countries. Symptoms are often debilitating and typically include pyrexia, severe abdominal pain and diarrhea (Walker et al., 1986).

Clostridial diseases progress rapidly and sudden death is often the first and the only sign of disease. *C. perfringens* type C causes necrotic enteritis in newborn calves. Calves are suddenly depressed, weak, and may be distended or show abdominal pain. If diarrhea develops, it may have blood and tissue streaks. Affected calves may die before they develop diarrhea (Quinn et al., 2002).

Bacterial isolation

In feces samples, microscopy will show gram negative rods, with no particular cell arrangement. Then, either MacConkey agar or EMB agar (or both) are inoculated with the feces. A typical *E. coli* were identified by their characteristic colony morphology on MacConkey's agar, biochemical characteristics and by a slide agglutination test with rabbit antiserum to the atypical *E. coli*. On MacConkey agar, deep red colonies are produced as the

organism is lactose positive, and fermentation of this sugar will cause the medium's pH to drop, leading to darkening of the medium. Growth on Levine EMB agar produces black colonies with greenish-black metallic sheen. The diagnosis of *E. coli* is also lysine positive, and grows on TSI slant with a (A/A/g+/H₂S-) profile. Also, the pattern of *E. coli* on, Indole, Methyl Red, Vagau's prousskare, and Citrat utilization is indole positive (red ring) and methyl red positive (bright red), but VP negative (no change-colorless) and citrate negative (no change-green color). Tests for toxin production can use mammalian cells in tissue culture, which are rapidly killed by shiga toxin. Although sensitive and very specific, this method is slow and expensive (Paton and Paton, 1998). Typically diagnosis has been done by culturing on sorbitol-MacConkey medium and then using typing antiserum. However, current latex assays and some typing antiserum have shown cross-reactions with non *E. coli* O157 colonies. Furthermore, not all *E. coli* O157 strains associated with HUS are non-sorbitol fermenters.

Diagnosis of salmonellosis involves isolation of *Salmonella* species in fecal cultures should be submitted for *Salmonella* culture in all cases of diarrhea or fever. Because *Salmonella* can be shed intermittently, repeated negative cultures must be obtained before ruling out salmonellosis. *S. typhimurium* recovered from calves and lambs were tested for their virulence using Congo red binding test, ability to produce hemolysin, adherence assay and cell invasion test and detection of inv A gene using PCR (Mohamed and Dapgh, 2007).

Accurate diagnosis of *Salmonella* require cultivation on specific media bacteriological testing of feces was undertaken for *Salmonella* using rapport and selenite brilliant green enrichment broths and sub-inoculation onto specific media such as; XLD, RVS, brilliant green agar, triple sugar iron agar, gram stain show medium sized gram negative rods and biochemical tests using API 20. Carriers of infections can be detected by culturing faces but because excretion is intermittent, repeated sampling and culture may be necessary. Serology may be useful but is best applied on a herd basis (Davies, 2008). No practical serological method exists for detecting individual carrier animals (Hansen et al., 2006).

For isolation of *Campylobacter*, a small portion of fecal samples was suspended in 0.85% saline, filtered through 0.45 mm millipore filter papers. Filters were then cultured in Preston broth and incubated overnight at 37°C.

Cultures were then inoculated onto Preston agar plates and incubated for 48 h in an atmosphere of 5% oxygen, 10% CO₂ and 85% nitrogen. Suspected colonies were identified based on their motility, hydrolysis of sodium hippurate and sensitivity to cefalotin and nalidixic acid (Achá et al., 2004). According to Klein et al. (2012), fecal samples were enriched in Bolton broth for 48 h at 42°C under microaerophilic conditions (10% CO₂, 5% O₂ and 85% N₂). A loopful of this enrichment was streaked onto modified charcoal cefoperazon deoxycholate agar and a

second loopful onto CampyFoodAgar. Both plates were incubated at 42°C for 48 h under microaerophilic conditions. Additionally, fecal material without prior enrichment was directly streaked on modified charcoal cefoperazon deoxycholate agar and Campy Food Agar, and incubated at 42°C for 48 h. Morphological typical colonies were differentiated by aerobic incubation.

For clostridia, samples should be collected in cooked meat broth or thioglycollate broth media, and then aerobically incubated. Gram staining illustrated gram positive spore forming rods. Biochemical analysis is required to differentiate between different species, such as cultivation onto Egg Yolk agar for lecithinase and lipase activity, testing for hydrolysis of gelatin, digestion of casein, indole production and formation of acid from glucose-lactose-sucrose maltose fermentation test. Animal inoculation test by intramuscular injection in mice or guinea pig for toxin identification by neutralization test using polyvalent antitoxin, followed by specific monovalent antitoxin. *C. perfringens* is also identified by Nagler test and CAMP test. Florescent antibody technique for identification of *Clostridium novy* in acetone fixed liver impression smear (Quinn et al., 1994).

Molecular diagnosis

Diagnosis of *E. coli* infection currently relies on the phenotypic differentiation of pathogenic strains from nonpathogenic normal flora *E. coli* via bioassays or immunoassays for toxins and fimbriae. As with bacterial classification, identification of bacteria is increasingly using molecular methods. Diagnostics using such DNA-based tools, such as polymerase chain reaction, are increasingly popular due to their specificity and speed, compared to culture-based methods (Louie, 2000). These methods also allow the detection and identification of "viable but non culturable" cells that are metabolically active but non-dividing (Oliver, 2005). Detecting *E. coli* O157 in feces include ELISA tests, colony immunoblots, direct immunofluorescence microscopy of filters, as well as immunocapture techniques using magnetic beads (De Boer and Heuvelink, 2000). These assays are designed as screening feces to allow rapid testing for the presence of *E. coli* O157 without prior culturing of the feces specimen.

Polymerase chain reaction followed by a microarray hybridization step has been used for the detection and typing of *E. coli* virulence genes (Chizhikov et al., 2001). A serotype-specific DNA microarray for the identification of clinically encountered Shigella and pathogenic *E. coli* strains were recently described (Li et al., 2006).

Diagnostic microarrays based on the ArrayTube format were devised for virulence determinant detection as well as for protein-based serotyping of *E. coli* (Anjum et al., 2007). A novel ArrayTube assay, which incorporates oligonucleotide DNA probes representing 24 of the most epidemiologically relevant O antigens and 47 H antigens,

has been described for fast DNA serotyping of *E. coli* (Ballmer et al., 2007). Serotyping is required and the use of mPCR is a useful accurate tool to detect toxic genes; shiga toxin and intamin that are responsible for signs of toxicity (Ahmed et al., 2007).

Various *Salmonella specific* primers and probes have been developed for molecular identification as well as detection in samples of these microorganisms. The use of pooled *Salmonella* enrichment broth cultures of bovine feces and PCR for the detection of the *invA* gene of *Salmonella* in feces appears to be an efficient method of *Salmonella* detection (Singer et al., 2006). *S. typhimurium* recovered from calves and lambs were tested for their virulence using Congo red binding test, ability to produce hemolysin, adherence assay and HEP2 cell invasion test and detection of *invA* gene using PCR (Mohamed and Dapgh, 2007). *Campylobacter* PCR (Linton et al., 1997) and 16S rRNA gene sequencing on selected *Campylobacter* isolates were further identified and differentiated by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry analysis as described by Alispahic et al. (2010). For MALDI-TOF, colonies were grown on Columbia blood agar containing 5% sheep blood at 42°C for 48 h under microaerobic conditions. Single colonies were removed and analyzed. *C. perfringens* toxins were detected using quantitative culture followed by genotyping. Toxin detection can be performed by several techniques including an enzyme linked immunosorbent assay (ELISA) that detects CPA, CPB, ETX and *C. perfringens* (Uzal and Songer, 2008).

In vitro methods for detection of toxinogenic are based on detection of toxins or gene probes or Multiplex-PCR for detection of toxin genes. The use of gene probes and multiplex PCR assays for detection of toxigenic *C. perfringens* in affected animals has also been reported (Baums et al., 2004). Multiplex PCR assays have been highlighted as a rapid and accurate method for the detection and genotyping of *C. perfringens* in routine veterinary diagnostics, and provide a useful alternative to *in vivo* toxin neutralization tests for typing of *C. perfringens* isolates (Meer and Songer, 1997).

Serological diagnosis

Cho (2012), described that recently a commercial antigen-capturing ELISA kit in form of a dipstick (Bovine Entericheck, Biovet) was made available to bovine practitioners and producers for the rapid detection of *E. coli* K99+ in feces from diarrheic calves at acute stage of clinical disease with diagnostic sensitivity and specificity of 71.4 and 100% respectively in comparison to mrt PCR multiplex real-time polymerase chain reaction. Serology may be useful for the diagnosis salmonella but is best applied on a herd basis (Davies, 2008). No practical serological method exists for detecting individual carrier animals (Hansen et al., 2006). Serotyping used slide

agglutination test and antibiotic sensitivity test for detection of R factor plasmid (Quinn et al., 1994). Enterotoxins of *C. perfringens* have been detected by enzyme-linked immunosorbent assay (ELISA), immunoelectrophoresis, latex agglutination and immunodiffusion (EL-idrissi and Ward, 1992).

Zoonotic importance

Increasingly, food animals and their products are being identified as important sources of infectious pathogens for humans. Many studies also showed that both healthy and diarrheic calves harbor STEC in their intestine (Roopnarine et al., 2007) and shed the bacteria for several months and in great quantities (Widirasih et al., 2004). In addition to economic losses, diarrhea in livestock is important because of the public health implications.

Numerous infectious agents causing diarrhea in animals are zoonotic and have been associated with food-borne diseases (Trevejo et al., 2005). Sporadic cases or large STEC outbreaks in humans are associated with the consumption of raw or undercooked meat of food animals and other foods contaminated by animal faces, and by contact with STEC-positive animals or with their environment (Paton and Paton, 1998).

Dairy and beef cattle are primary reservoirs of *E. coli* O157:H7 and they can carry it asymptotically and shed it in their feces (Bach et al., 2002). Food products associated with *E. coli* outbreaks include raw ground beef and raw seed sprouts or spinach (Sabin, 2006) raw milk, unpasteurized juice, unpasteurized cheese and foods contaminated by infected food workers via fecal-oral route. *E. coli* and their subtypes (O26, O111, O118 and O157) are firmly associated with emergent food-borne diseases, especially Shiga toxin-producing *E. coli* (STEC).

In humans, EHEC a subset of STEC, is associated with severe systemic disease as haemorrhagic colitis (HC), haemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), especially in infants, young children and in the elderly (Nataro and Kaper, 1998).

Cattle have been identified as the main animal source of *S. typhimurium* infections in humans potentially, spread from cattle to humans can be via direct contact, contamination of the environment and contamination of meat and milk products. Milk has been reported as the source of human infection (Kavanagh, 2002). *C. perfringens* bacteria are the third-most-common cause of food-borne illness, with poorly prepared meat and poultry the main culprits in harboring the bacterium (Warrell et al., 2003).

The *C. perfringens* CPE mediating the disease is heat-labile (dies at 74°C) and can be detected in contaminated food, if not heated properly, and feces (Murray, 2009)

Control and prevention

Multiple factors, both infectious and non-infectious, are involved in calf diarrhea outbreaks, which makes disease control on farms difficult (Svensson et al., 2003; Trotz-Williams et al., 2007). Timely prevention and control of calf diarrhea is important to reduce economic losses to producers and improve animal welfare (McGuirk, 2008). Dealing with such a large number of potential etiological agents as well as various management factors, is an ongoing challenge for effective control of enteric disease in newborn calves (Booker et al., 2008; Uhde et al., 2008). Therefore, a thorough investigation should include a study of dry cow management and calving practice as well as calf management (Bazely, 2003; Andrews, 2004).

Studies of dairy herds have indicated that improved environmental management of calving pens and calf housing reduces calf diarrhea incidence and the extent of outbreaks. A system of "all in all out" calf housing, cleaning, steaming and disinfection of calf housing and calving pens, regular disinfection of utensils and adequate straw were identified as important management factors. In a study, calf diarrhea incidence was reduced from 36 to 11% within a year by the introduction of early colostrum feeding and improved housing hygiene (Lance et al., 1992).

The passive immunity acquired from the colostrum and absorbed into the circulation from the gut is the calf's main defense mechanism against *E. coli* diarrhea. Inadequate amounts of immunoglobulins in the colostrum, inadequate intake of the colostrum and inadequate absorption of immunoglobulins from the gut render very young calves susceptible to infection (Groutides and Michell, 1990). Additionally, among calves aged 1 to 4 months old, carriage of VTEC *E. coli* O157 was reduced if the calf had suckled colostrum from the mother or if the calf had stayed more than 2 days with the mother after calving (Rugbjerg et al., 2003).

The eradication and control of salmonellosis on a persistently infected farm is difficult. Fecal or serological sampling of all animals with group and individual samples, and identification and slaughter of carrier animals has been used successfully in voluntary salmonellosis control (Jensen et al., 1994). Combined vaccination, fecal identification and culling of infected animals have also been used with varying success (Davies and Renton, 1992). Intensive care with antitoxin, fluids, antibiotics and anti-inflammatory drugs is necessary for treatment but frequently unsuccessful (Quinn et al., 2002).

Synergism between proplis and antibiotics for treatment of enterotoxaemia in calves due to *C. perfringens* type A and C show great results (Masoud et al., 2008). Commercial toxoids available for vaccination against *C. perfringens*, is not effective against type E infections (Songer and Miskimmins, 2004). Clostridia mode of action is to produce one or more potent toxins. Therefore,

the best program is obtained by the use of toxoid vaccines, it allow protection to pass to the lamb via the colostrum (Lewis, 2011).

Treatment of calf diarrhea

In case of diarrhoea outbreak in a herd, it is important to attempt to diagnose the infective cause of the disease in order to target further control measures appropriately. Isolation of affected calves, effective treatment with rehydration solutions and provision of dry and warm conditions are vital in the treatment of calf scours. The main aim of treatment is to restore the fluid balance in the animal by rehydration therapy. Oral therapy is the best way of providing rehydration fluids, but this may need to be replaced by intravenous therapy in severe cases where the calf is unable to drink. A return to whole milk feeding is recommended within two days of rehydration therapy to avoid a negative energy balance (Grove-White, 2004). Suckled calves should have limited access to the dam or sucker cow until full recovery has been achieved.

The use of antibiotics in diarrheic calves has been shown to be contraindicated in many studies, due to the further disruption of gut flora, the establishment of carrier states of salmonella-infection, and the development of antimicrobial resistance factors in the enteric flora. Although, very sick calves with *Salmonellosis* may benefit from antimicrobial therapy, most studies have also failed to show any beneficial effect of antimicrobial treatment (Rollin et al., 1986). Some studies show limited efficacy in reducing mortality and morbidity in an outbreak of diarrhoea (Holck et al., 1994).

Antibiotics should only be used for *E. coli* and *Salmonella* infection, after sensitivity test to choose the best drug, as inappropriate use of antibiotics can lead to serious antibiotic resistance problems. Ciprofloxacin and probiotics such as *Lactobacillus acidophilus* isolated from colostrum of goat and mere are highly effective in treatment of infection thus, control of calf scour is based on feeding plenty of colostrum immediately after birth (Abd El-Moez et al., 2010). Ciprofloxacin coated with gold nanoparticles showed high hindrance *in vitro* for the growth of *E. coli* and *S. typhimurium* (Zawrah and Abd El-Moez, 2011). Vaccination is very important in the control of calf scour, vaccines are to protect against *E. coli* and rotavirus. However, it is unlikely to be effective unless used in conjunction with good husbandry (Hirsh and Zee, 1999).

The treatment of *Salmonella* infection in cattle with antibiotics in acute cases is common and may reduce mortalities if initiated early and combined with support therapy. The use of antibiotics metaphylactically in the face of an outbreak is not recommended, due to the high risk of antimicrobial resistance development. Prophylactic antibiotic administration in feed does not appear to have

any effect on excretion of *Salmonellae* in calves either in an outbreak, appropriate support therapy for severely affected animals and vaccination can be helpful.

CONCLUSIONS AND RECOMMENDATIONS

Most cases of calf diarrhea are likely to be mixed infections, where more than one of the pathogenic agents is present. The impacts of calf diarrhea could be direct by causing calf deaths and indirect through increased treatment expenses and decreased lifetime productivity and survivorship. Among the bacterial causes of diarrhea in neonatal food animals, *E. coli* and *Salmonella* specie are the most common and economically important but, *C. perfringens*, and *Campylobacter* species have also been identified as causes of enteric diseases in calf diarrhea. Diarrhea in calves is commonly caused by ETEC; more recently, AEEC and STEC have also been identified as causes of diarrhea and dysentery in calves.

Acute diarrheal disease is most common with *S. typhimurium* and systemic disease with *S. dublin* in cattle. *Campylobacter* specie, especially *C. jejuni*, may cause diarrhea in calves. However, newborn calves which have a low level of proteolytic enzymes in gastrointestinal tract can be easily infected by *C. perfringens* type C. Diarrhea in livestock is important because of the public health implications. Numerous infectious agents causing diarrhea in animals are zoonotic and have been associated with food-borne diseases. Inadequate intake of colostrum, poor hygiene, overcrowding in the calf pens, low temperature of the incoming air, contamination of the incoming air, inadequate ventilation, close proximity to adult cows, mixing of different age groups are the major risk factors for calf diarrhea.

In Ethiopia, very few studies were carried out on the identification of the causes of calf morbidity and mortality giving special emphasis on the cause of calf diarrhea in dairy farms of the country. It is important to identify the infectious agents in the outbreaks of calf diarrhea on a farm basis in order to target prevention and control of this disease complex, and implementation of improved calf management practice to reduce the high-level risk factors of calf disease problems. Detailed studies on the extent of calf diarrhea in Ethiopia, on its etiology and risk factors for infection and zoonotic significance of these agents should be initiated.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Full Length Research Paper

Prevalence of small ruminant trypanosomosis in Assosa and Homosha districts, Benishangul Gumuz Regional State, North West of Ethiopia

Zelalem Ayana¹, Diriba Lemma^{2*}, Birhanu Abera² and Eyob Eticha²¹Jimma University college of Agriculture and veterinary medicine, Ethiopia²Asella Regional veterinary laboratory, Ethiopia

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A cross sectional study was conducted at Assosa zone of Benishangul Gumuz, North West Ethiopia, to determine the prevalence of trypanosomosis in local breeds of sheep and goats. Blood sample from 384 randomly selected sheep and goats (177 from Assosa and 207 from Homosha districts) of different species, sex, age groups were examined by dark phase contrast buffy coat and thin smear examination for species identification of trypanosome. Among the animals examined during the study period, 10 (2.6%) were infected with trypanosomes. From this survey, *Trypanosoma vivax* was found to be the major cause of trypanosomosis (1.82%), followed by *Trypanosoma congolense* (0.52%) and *Trypanosoma brucei* (0.26%). There was no statistical difference ($p > 0.05$) in infection between sex, species, and among age groups. Mean packed cell volume (PCV) of the parastemic animals was significantly lower than ($P < 0.05$) that of aparastemic animals. In attempt to identify the vector involved in transmission, tsetse flies group (*Glossina morsitans submorsitans*) and mechanical vectors of trypanosomosis that belonging to Tabanidae (*Tabanus*, *Stomoxys* and *Haematopota*) were captured in both districts at an altitude range of 1270 to 1507 m above sea level. The results of the prevalence of the disease in small ruminants and its vectors indicate that an effective management and control measures for the disease and transmitting vectors should be designed and implemented.

Key words: Assosa, small ruminant, trypanosomosis, prevalence, vector.

INTRODUCTION

Trypanosomosis is a major constraint on ruminant livestock production in many areas of Africa including Ethiopia. Many animal species can be affected by the different trypanosomes, thus severely impairing the economic efficiency in endemic areas. From an economic point of view, the disease is particularly important in cattle, although other mammals can also be affected (Gupta et al., 2003; Singla et al., 2009; Sharma et al.,

2012; Kumar et al., 2012). Although geographical differences have been observed, but the most affected animal species are buffalo, horses, camels, cattle and dog (Singh et al., 2003; Singla et al., 2012; Sharma et al., 2013; Sumbria et al., 2014). Other hosts, including small ruminants, can be affected (Corbera et al., 2006).

Trypanosomosis is a parasite disease caused by species of flagellated protozoa belonging to the genus

*Corresponding author. E-mail: lemmadiriba@yahoo.com.

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trypanosome which inhibit the blood plasma, various blood tissues and liquid of vertebrate host (Rodostitis et al., 2000; Juyal et al., 2005). The infection caused by different species of trypanosomes is mainly transmitted cyclically by tsetse flies and mechanically by biting flies belonging to families Tabanidae and Hippoboscidae if they feed on more than one host within a short interval (Radostits et al., 2000; OIE, 2008). It is a serious constraint to agricultural production in extensive areas of the tsetse-infested Ethiopian lowlands (Slingenbergh, 1992). Out of the nine regions of Ethiopia, five (Amhara, beneshangul-Gumuz, Gambella, Oromiya and SNNPR) are infested with more than one species of tsetse flies (NTTICC, 2004).

The epidemiology of typanosomosis depends on factors such as distribution of vectors, virulence of parasite and response of host. The disease follows the distribution and intensity of various species of tsetse fly (Bursell, 1960). The most important tsetse flies species that are distributed and infested parts of Ethiopia are *Glossina morsitans submorsitans*, *Glossina pallidipes*, *Glossina fuscipes fuscipes*, *Glossina tachinoides* and *Glossina longipennis* (Langridge, 1976; Getachew and Hunduma, 2005). Sheep and goats are naturally infected with *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma brucei* and *Trypanosoma evansi* which produce acute, subacute, chronic, or subclinical forms of disease in these animals (Getachew, 2005; Corbera et al., 2006). Even though small ruminants are naturally infected and presenting clinical disease, it is commonly believed that they are highly resistant to infection, but it is only sporadic, and that the disease in these animals is of little economic consequence (Corbera et al., 2006). The role of small ruminant in epidemiology of the disease in nature is still not well known. But the current epidemiological information indicates that the sheep and goats could act as reservoirs for the spread to animal and human trypanosomosis (Dede et al., 2005).

In view of this, the present study was designed to estimate the prevalence rate of typanosomosis in small ruminants, identify species of trypanosomes, determine the packed cell volume (PCV) values on infected and non infected animals and the vector density tsetse and other biting flies of the study area.

MATERIALS AND METHODS

Study area

This study was conducted in two districts (Assosa and Homosha) of Assosa zone of Benishangul Gumuz Regional State. The study was limited to only two peasant associations from each district due to their scatteredness and limited resources. These are Ashura and Bamadon from Homosha district; Ura and Tsetse Adurnon from Assosa district. Assosa is found at 675 km far from capital city, of Addis Ababa, North Western part of Ethiopia at 34° 02' 20E to 36°30'E and 9°30'N to 11°39'N. The maximum and minimum temperature of the area ranges from 35 to 25°C, respectively with the altitude ranging from 600 to 2731 m above sea level and the

mean annual rain fall of 1000 mm. The area was gaining the rain for six month duration which starts on April and ends on October. The main occupation of the population is mixed farming practice of crop and livestock. The major livestock reared in the area are bovine, sheep, goats, donkeys and poultry (RSA, 2007). According to the publication bureau of Assosa Zone Agricultural office, the number of animal population in the area were 39133 Cattle, 6977 Sheep, 17675 Goat and Equines 2558 in Assosa district and 3665 cattle, 1350 Sheep, 3752 goat and 317 Equines in Homosha district.

The vegetations type of the area is savanna grass with scattered trees of different species dominated by bamboo trees that covers large area of the region with fat low land. Among the wild game few of them are antelope, bush pig, baboons and warthog (NTTICC, 2004).

Sample size

The sample size was determined using Thrusfield (2005) formula. Totally, 384 samples were collected from four sites of the two districts, namely, Ashura, Bamadon, Ura and Tsetse Adurnon by simple random sampling method.

Study animals

The study was conducted on 384 local breeds of the sheep and goats. From each selected peasant association, a proportional number to the total population of the study animals were sampled. The sample size were 86, 121, 58 and 119 in Ashura, Bamadon, Ura and Tsetse Adurnon, respectively. Examination and evaluation of body condition were accomplished during sample collection. They were classified as very thin (1), thin (2) moderate(3), good(4) and very good (5) by observing the body condition of animals in the field (Cooper and Thomas, 1985). The age of animal were also estimated by examining dentations (Kripali et al., 2010) and information obtained from owner.

Study design

The study was based on entomological and parasitological survey. This cross-sectional study were conducted in two districts.

Parasitological and hematological examination

Blood sample were collected randomly from small ruminants of four sites during the study period. Blood was collected from ear vein using sterile blood lancet and capillary tube. Pair of heparinized capillary tube were filled with blood from small ruminants to 3/4 of its height and sealed at one end with crystal seal. The capillary tube were loaded on the microhaematocrit centrifuge symmetrically and centrifuged at 12000 rpm for 5 min (Murray et al., 1997). Packed cell volume (PCV) was determined using Haematocrit reader (Woo, 1970). After the PCV has been read, capillary tubes were broken in 1 mm below the Buffy coat which includes red blood cell layer and the content were expressed on microscopic slide and covered with a 22 × 22 mm cover slip. The content was examined under ×40 objective using dark ground Buffy coat technique (Murray et al., 1997). From positive sample, thin smear were made, fixed with methanol for 5 min and stain with geimsa solution for 30 min and examined using oil immersion under × 100 objective to detect the species of trypanosome.

Fly survey

The survey was under taken in two districts of Assosa zone in four

peasant associations. A mono- pyramidal trap was used, to trap different flies, total of 48 h were used and 59 traps were deployed. The collected flies' were counted and identified using hand lenses.

Mono-pyramidal traps baited with acetone, octenol, and cow urine (Brightwell et al., 1987) were used for assessing the fly density. The site selection was to include suitable tsetse habitats like savanna area, river valleys, livestock grazing area and watering points and vicinity to assume wild game reserve area. In all the study sites, a total of 59 mono-pyramidal traps were deployed early in the morning and maintained in position for 48 h. Savanna tsetse (*Glossina morsitans sub morsitans*) can detect odour from about 200 m. So, the traps were spaced at about 200 m interval.

During trapping, acetone and octenol were dispensed from open vials through an approximately O-size hole. While cow urine from open bottle on a piece of tissue paper was included to facilitate odor diffusion. All odors were placed on the ground, above 20 cm up the wind of the trap. The traps poles were greased to exclude insect predators like ants. The different catches in the traps were counted, identified (Langride et al., 1976) and analyzed. The species of tsetse fly was identified based on characteristic morphology (Leak et al., 1993). Other biting flies are separated according to their morphological characteristic, such as size, color, wing venation structure and proboscis at genus level (Wall and Shearer, 1997).

Sexing was done just by observing the posterior end of the ventral aspect of the abdomen by hand lenses. As a result, male fly is easily identified by enlarged hypopygium in posterior ventral part of the abdomen (Challier, 1965). The fly apparent density is the mean caught in traps deployed, expressed as the number of fly caught per trap per day (Leak, 1999).

Data analysis

Statistical analysis was employed by Chi-square (χ^2) for data management and analysis using Stata Version 7.0 (2000). The tested hypothesis were prevalence of trypanosomes, PCV value, the relation between PCV value and prevalence of trypanosomes, the relation between age value and prevalence of trypanosome.

RESULTS

During this study, a total of 384 small ruminants of local breeds were examined in both study areas, out of these 207 were from Homosha and 117 from Assosa district. Generally, the overall prevalence rate of the two districts was 2.6% (Table 2.)

The trypanosome species encountered are *T. vivax*, *T. congolense* and *T. brucei*. *T. vivax* and *T. congolense* were found in two districts, but *T. brucei* was in Homosha only. The relative proportion of trypanosome species were 1.82, 0.52 and 0.26% for *T. vivax*, *T. congolense* and *T. brucei*, respectively (Tables 1 and 3).

There was no statistically significant difference ($P>0.05$) between trypanosome infection rates in the village of two Woredas; with prevalence rates of 3.83, 1.64, 3.45 and 2.52% in Ashura, Bamadon, Ura and Tsetse Adurnon, respectively (Table 4).

Rate of infection was 3.82 and 1.78% in ovine and caprine, respectively with an overall infection rate of 2.60%. There is no significant difference ($P>0.05$) above different species of small ruminant (Table 5).

The body condition of all sampled small ruminant was also evaluated by scoring method indicated by Cooper and Thomas (1985). Out of the infected small ruminant, 3.85% were with thin body condition, 2.68% with moderate body condition, and 2.06% with good body condition (Table 6).

Infection rate was 0 and 2.98% in less than two years and greater than two and/or equal to two, respectively (Table 7). There is no statistically significant difference ($P>0.05$) in different age groups.

Infection rate between different sexes were 2.25 and 2.9% for male and female, respectively (Table 8). There is no statistically significant difference ($p>0.05$) in different sex groups.

There was statistically significant ($P<0.05$) difference in means PCV of parastemic and aparastemic small ruminants. The mean PCV value of parastemic and aparastemic were 20.9 and 27.95%, respectively (Table 9).

DISCUSSION

The results indicated that trypanosomosis to be the important livestock disease in Assosa zone of North West Ethiopia as also reported by Tewolde et al. (2001) in Metekel district, North West of Ethiopia. Even though various conventional diseases induce livestock mortality and results in economic losses in Ethiopia, tsetse transmitted trypanosomosis has a crucial effect which is becoming unchallengable to treat. Vector control action as a strategy option is not widely implemented in Ethiopia (NTTICC, 2004).

The finding of tsetse survey revealed one type of tsetse species at Homosha and Assosa districts Woreda. The main vector were *G. m. submorsitans* which was similar with the previous result in Metekel district (Getachew and Hunduma, 2005). Typical habitat was found in the study area for savanna species, that is, *G. m. submorsitans* prefer savanna grass, riverine and forest ecology. *G. m. submorsitans* was found to be concentrated in low land areas as climatic condition were more favourable. Some flies however, were found as high as 170 m. Earlier works had established the tsetse geographical limit at 1600 m. Later, Slingenbergh (1992) found the increased limit up to 2000 m.

During the tsetse survey in two Woredas of the Assosa zone 0.0086 and 0.018 flies/trap/day were captured in Homosha and Assosa districts, respectively. Small fly density was obtained because of dry season. There were uncontrolled bush fires few weeks prior to the survey period. Such circumstance might have suppressed the fly density and forced the fly to move the moisture area and river banks of extreme low altitude. The fly density was found to be relatively increased in late rainy season than dry season. This fact is in agreement with result of Leak et al. (1993). According to Leak et al. (1999),

Table 1. Species and number of vectors identified at two districts of Assosa zone.

Village	Altitude (m)	No. of traps	Types and species of flies	Sex		Total	FTD
				Male	Female		
Ashura	1270-1352	14	<i>G. m. submorsitans</i>	1	-	1	0.0015
			<i>Stomoxys</i>	-	-	15	0.022
			<i>Tabanus</i>	-	-	6	0.00089
			<i>Haematopota</i>	-	-	8	0.0119
Bamadon	1350-1410	15	<i>G. m. submorsitans</i>	4	7	11	0.01553
			<i>Stomoxys</i>	-	-	29	0.04202
			<i>Tabanus</i>	-	-	24	0.0333
			<i>Haematopota</i>	-	-	1	0.0014
Ura	1390-1418	15	<i>G. m. submorsitans</i>	1	-	1	0.0014
			<i>Stomoxys</i>	-	-	48	0.0666
			<i>Tabanus</i>	-	-	4	0.00555
			<i>Haematopota</i>	-	-	-	0
Tsetse Adurnon	1460-1507	15	<i>G. m. submorsitans</i>	6	19	25	0.034
			<i>Stomoxys</i>	-	-	122	0.1694
			<i>Tabanus</i>	-	-	14	0.0194
			<i>Haematopota</i>	-	-	5	0.0069

Table 2. Prevalence small ruminant trypanosomosis on district basis.

District	No. of animals examined	Positive animals	Prevalence rate (%)	χ^2 cal	P value
Homosha	207	5	2.41		
Assosa	177	5	2.82	0.0592	0.808
Total	384	10	2.6		

Table 3. Relative proportion of different trypanosome species on basis of district.

District	Positive animals	<i>T. vivax</i>	<i>T. congolense</i>	<i>T. brucie</i>	χ^2 cal	P value
Homosha	5	3	1	1		
Assosa	5	4	1	-	1.2087	0.751
Infection rate (%)	-	1.82	0.52	0.26		

Table 4. Prevalence of small ruminant trypanosomosis on the basis of the study peasant associations.

Peasant association	Examined animals	Positive animals	Prevalence rate (%)	χ^2 cal	P value
Ashura	86	3	3.53		
Bamadon	121	2	1.64		
Ura	58	2	3.45	0.901	0.825
TsetseAdurnon	119	3	2.52		
Total	384	10	2.6		

Table 5. Prevalence of small ruminant trypanosomosis based on species.

Animals species	Examined animals	Positive animals	Prevalence rate (%)	χ^2 cal	P value
Ovine	157	6	3.82		
Caprine	227	4	1.76	1.552	0.213
Total	384	10	2.6		

Table 6. Prevalence of small ruminant trypanosomosis on the basis of body condition

Body condition	Examined animals	Positive animals	Prevalence rate (%)	χ^2 cal	P value
Thin	78	3	3.84		
Medium	112	3	2.68		
Good	194	4	2.06	0.059	0.704
Total	384	10	2.6		

Table 7. Prevalence of small ruminant trypanosomosis on the basis of age.

Age category	Examined animals	Positive animals	Prevalence rate (%)	χ^2 cal	P value
<2 years	48	0	0		
≥2 years	336	10	2.98	0.059	0.809
Total	384	10	2.6		

Table 8. Prevalence of small ruminant trypanosomosis on the basis of sex.

Sex	Examined animals	Positive animals	Prevalence rate (%)	χ^2 cal	P value
Male	178	4	2.25		
Female	206	6	2.9	3.215	0.36
Total	384	10	2.6		

Table 9. Mean PCV value of parastemic and aparastemic small ruminant.

State of the animal	No. of animals	Mean PCV	Minimum	Maximum	SD	% Total N	χ^2 cal	P value
Parastemic	10	20.90	17	25	0.72	97.4		
aparastemic	174	27.95	18	37	0.14	2.6	196.126	0.000
Total	384	-	-	-	-	-	-	-

increased in fly density was due to the growth of vegetation and formation of new habitat in the rainy season.

There was no significant difference ($p > 0.05$) in infection rate among thin, medium and good body condition. This result is similar to the work of Goossens et al. (1998) and Snow et al. (1996). These researchers have indicated that small ruminant are not often selected by tsetse flies. Though these animals do not show signs of trypanosomosis. The same result is revealed by Getachew and Hunduma (2005) at South West of

Ethiopia and report that trypanosomosis in sheep and goats is an important disease and small ruminant serve as potential reservoir of infection for other animals and do not show clinical sign and were in good body condition.

The present study revealed that when the age increased, the prevalence rate also increased. This may be due to the immune compromization in very old individuals. The findings from Muturi (1999) and Terzu (2004) support the present finding. Out of the three detected trypanosomes, *T. vivax* stands first and this is due to the fact that its ability is being transmitted by

mechanically as well as cyclically and there is also little number of *Glossina* species when compared with other biting flies. This result is similar to the work of Kalu and Uzoigwe (1996), who reported that the area encountered low density of tsetse flies and *T. vivax* was a predominant species. This suggests that the biting flies would mediate *T. vivax* infection when tsetse fly density is low or absent.

There was no significant difference ($p>0.05$) in the infection rate among species of small ruminant, because in this study, area management, grazing area and nutritional status of the two species were the same. The same result is revealed by Coulibaly et al. (1995) and Defly et al. (1988) indicates that livestock species had a major effect on trypanosome prevalence.

It is known that the development of anemia is the most reliable indicator of the progress of trypanosome infection (ILRAD, 1998). But it can also be assumed that numerous concurrent diseases and nutritional factors interferes with anemic development (O.A.U/S.T.R.C., 1979) and PCV value are reliable indicator of anemia. During PCV determination, a value of 24 to 46 (Radostitis et al., 2000) was considered to be normal.

The mean PCV values of parastemic (20.9%) was found to be statically lower than ($p>0.05$) that of aparastemic (27.95%) small ruminant. Similar result were obtained at South West Ethiopia by (Getachew and Hunduma, 2005). Taylor (1998) indicated that the anemia persists during chronic infection of when parastemia is quite low, probably because of different mechanisms that are involved in its genesis during acute and chronic stage of infection (Singla et al., 1997). Thus suggest that control of parastemia is unrelated in chronic phase when immune system is depressed and anemia is sustained through dyserythropoiesis.

Conclusion

Trypanosomosis was prevalent in small ruminant in the study area at low rate of 2.6% with different trypanosome species. Prevalence of mechanical transmitters or biting flies such as *Stomoxys*, *Tabanus* and *Haematopota* along with cyclically tsetse *G. m. submorsitans* species indicated the vectors for trypanosomes in the study area. Regarding the trypanosomosis in small ruminant, a severe clinical signs does not overt by infection, but they can be considered as an important reservoir for the majority of trypanosomes for other animals and humans. Therefore, in areas where the presence of small ruminants are important and trypanosomosis is prevalent, these animals should be taken into consideration in all programs to control the disease.

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Conflicts of interest

Authors have none to declare

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Full Length Research Paper

Determinants of small ruminant farmers' decision to participate in veterinary services in Northern Ghana

Faizal Adams* and Kwasi Ohene-Yankyera

Department of Agricultural Economics, Agribusiness and Extension, College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology Kumasi, Ghana.

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This study analysed the determinants of small ruminant farmers' participation in veterinary services in Northern Ghana. Multi-stage sampling technique was used to collect data on 249 farm households in different locations in Northern Ghana. Analytical tools including frequencies, means and logistic regression model were used to analyse the data. The regression analysis indicates a positive relationship between participation in veterinary services and sex of household head ($p < 0.05$), education ($p < 0.05$), household income level ($P < 0.05$), herd size ($p < 0.05$), and affordability of veterinary service ($P < 0.01$). In addition, the study shows that diseases and pests menace, insufficient veterinary offices and animal health professionals were the major three constraints affecting animal health management in northern Ghana. In order to improve quality delivery of veterinary service in the area, the result of the logistic model provides a guideline to select farm households for implementation of veterinary extension programs in the region. Such guideline should be based on the important socio-economic and farm-related variables identified from the regression analysis. Furthermore, sustainable livestock production can be enhanced when animal health centers and professionals are made visible at local farming communities. Therefore, policies that provide an enabling environment for more private veterinary practice is relevant. More so, more qualified animal health professionals need to be trained to commensurate with the increasing number of livestock smallholders in the country.

Key words: Small ruminant, farm households, veterinary services, logistic regression, Northern Ghana.

INTRODUCTION

Small ruminant livestock (that is, sheep and goat) are widely distributed across Ghana (Mahama et al., 2003; Ockling, 1987). The West African Dwarf (WAD) or *djallonké* breeds are the most common nationwide, and often used in breed improvement schemes by individual farmers, or parastatal farms and breeding stations in

Ghana (Karbo et al., 1997b; Opong-Anane, 2006). Even though, the WAD breeds do not exhibit dwarfism traits (especially sheep), the animals are biologically adaptable to various vegetations, and demonstrates high typano-tolerance, high prolificacy and ability to breed all year round (Opong-Anane, 2006; Terril, 1985). Therefore,

*Corresponding author. E-mail: fazztop@yahoo.com. Tel: 233-268818687. Fax: 233-3220-60137.

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climatic conditions may have little or no effect on the animals' productive capacity, except changes in feed supply that can alter physical and physiological maturity of the animals (Addah and Yakubu, 2008).

Northern Ghana is the hub of livestock production in Ghana. Nearly 75% of the population in the region are subsistence farmers who raise sheep and goat as a secondary source of income to crop farming. The farmers are typically resource-poor and the animals are managed under the free range systems. As a result, use of feed supplements, veterinary healthcare, good housing or quality breeds tend to be limited (African Development Fund, 2001). Farmers' investment in small ruminant livestock is through purchase, inheritance or as gifts to replenish the farm stock (Suleman, 2006). The animals commonly scavenge for food and water around villages or homesteads without a stock herder during long dry seasons. In the wet season (cropping season), the animals are either tethered around homesteads or herded to communal areas for grazing by older or younger family members (Upton, 1984). Animal mortality rate is high under system mainly due to poor housing, overcrowding, inadequate veterinary stations (to supply drugs/medicines) and poor ventilation, resulting in diseases and parasites such as pneumonia and diarrhoea, especially during the rainy periods (Terril, 1985; Turkson et al., 2004).

The contribution of sheep and goat to food security and poverty reduction is under-exploited in Ghana, especially Northern Ghana (Mahama, 2012; Otchere, 1986). The animals are raised in vulnerable farm households not only for meat (sales), but also as an important source of wealth and savings, and as insurance against crop failure (Dossa et al., 2008; Otchere, 1986). For many subsistent farmers in Northern Ghana, small ruminants help to improve the animal protein requirements in the home. Sheep and goat have distinct advantages over other livestock in converting poor nutritious feed such as straw and grasses, as well as other by-products such as kitchen scrap and other waste products into value-added high quality food products for human consumption (IFAD, 2004; Terril, 1985). In addition, the meat of small ruminants is a source of protein in many local cereals-based diets and can improve the nutrition of vulnerable children and pregnant women (Terril, 1985). The size of small ruminants, which, on average, generate about 20 to 35 kg carcass weight (Oppong-Anane, 2006), allow rural households to conveniently process them easily for home consumption, with little or no need for preservation (Lebbie, 2004; Oluwatayo and Oluwatayo, 2012).

Notwithstanding the immense role of small ruminants in the lives and livelihoods of rural households, the production potential of the animals is limited by various factors in Ghana. Critical among such constraints is insufficient animal health care support (Turkson, 2003). The limited support for livestock health services has a negative effect on livestock productivity in the country

(Mahama, 2012). In fact, the annual economic loss associated with livestock mortality and diseases and pest outbreak is estimated at US\$50 million (Ministry of Food and Agriculture, 2007). The problem is chronic due to insufficient government budget allocation to animal health care systems in the country. Meanwhile, initiatives to promote growth of private veterinary service delivery is limited by numerous constraints, including poor government legislation to support privatization, the dominance of subsistent farmers (unable to pay for veterinary services), and a shortfall in well trained extension personnel (Turkson, 2003).

The Veterinary Services Directorate (VSD) of the Ministry of Food and Agriculture is responsible for animal health service delivery in Ghana. In addition, many private veterinary service providers have gained recognition since the country adopted the Structural Adjustment Policy (SAP) in the 1980s (Amankwah et al., 2014; FAO, 1999; Turkson, 2003). The importance of the animal health care systems is not only to sustain and improve livestock production through animal disease and parasite prevention, but also to protect humans against zoonotic diseases and infections (Amankwah et al., 2014; Okereke, 2012). Therefore, providing quality animal health care service is essential for efficient livestock production and quality animal products for human consumption (Turkson, 2008). However, the delivery of quality veterinary service remains a major challenge in developing countries (Gbolagade et al., 2013; Meena, 2013). An important reason for the limited success in quality veterinary service delivery is because factors that influence participation in veterinary services are not well known in sub-Saharan Africa (Onono et al., 2013). According to Posavac and Carey (1992) cited by Turkson and Amakye-Ansah (2005), effective services are delivered if only the services are consistent with the needs and objectives of the customer. However, livestock technical staff often places much emphasis on the technical aspects of production with little attention to the actual needs and objectives of subsistent farmers (Bosman, 1995; Schuetterle and Coulibaly, 1987). Various studies for sub-Sahara African countries (Dossa et al., 2008; Fakoya and Olurntoba, 2009; Mahanjana and Cronje, 2000; Verbeek et al., 2007), suggest that local farmers' production objectives and livelihood needs associated with managing livestock are influenced by social and economic factors, as well as farm-related variables. Even though, few studies (Turkson, 2003, 2008; Turkson and Amakye, 2005; Turkson and Naandam, 2003) have been conducted to describe veterinary service utilization among livestock farmers in Ghana, there is no comprehensive analysis of the veterinary needs of small ruminant livestock farmers that explicitly accounts for the effect of socio-economic and farm-related factors in farmer's decision to participate in veterinary services in Northern Ghana. However, studies in Nigeria (Adesope et al., 2006; Okereke, 2012) and

Ethiopia suggest that important farmer and non-farmer characteristics influence farmer's decision to participate in veterinary services. Such technical information is important for customizing and developing rural farmer-relevant veterinary service support programs.

The aim of this study was to investigate the determinants of subsistent small ruminant farmers' participation in veterinary services in Northern Ghana. One objective was to determine the effect of socio-economic and farm-related factors on participation in veterinary services among subsistent small ruminant farmers. An understanding of such factors can help in evaluating veterinary service intervention strategies for less productive farmers in Northern Ghana. In addition, another objective was to examine common livestock health problems that limit sheep and goat production in Northern Ghana. This includes comparing the problems between the two agro-ecological zones.

METHODOLOGY

Study area

The study was carried out in Northern Ghana which comprises of three government administrative regions, namely, Northern Region (NR), Upper West Region (UWR) and Upper East Region (UER). The area shares boundaries with Brong-Ahafo Region (BAR) to the south, Togo to the east, part of Cote d'Ivoire to the west and Burkina Faso to the north. Northern Ghana lies between latitude 80 to 110° N and longitude 00 to 30° W. The area covers a land mass of 97,700 km², equivalent to 38.7% of Ghana's total land area of Ghana (or 238,539 km²) (Ministry of Food and Agriculture, 2010).

Northern Ghana is made of up two agro-ecological zones, including Guinea Savannah (GS) and Sudan Savannah (SS) zone. The climatic conditions of the two zones are typified by high temperature ranges (24 to 38°C for GS and 25 to 36°C for SS), low rainfall amount (1100 for GS and 1000 for SS) and long spells of drought periods. Hence, the vegetative cover is arid in nature. Given this kind of vegetation, the dominant occupation in the area is peasant agricultural production, which includes livestock production (sheep, goat, cattle, pigs, chicken, donkey, among others), and crop production such as sorghum, yam, millet, maize, guinea corn, cassava, rice, cowpea and groundnuts (FAO, 2005; Karbo and Agyare, 1997a).

Research methods and sampling procedure

Both qualitative and quantitative research methods were used to collect data for the study. Quantitative data were collected through pre-tested survey questions. Specifically, data on farmer and farm-related variables were collected. In addition, common animal health problems affecting livestock production in the study area were also solicited. Focus group discussions (qualitative approach) were also held to gain more information and improve on the quality of data. Multi-stage sampling method was used to choose 300 farm households in the study area. Stage one includes purposive sampling of 3 districts under each region based on accessibility and logistic considerations to carry out the study. Then, 2 farming communities were randomly selected from each district under each region totalling 6 villages at stage two. Finally, 300 farm families were randomly selected based on a sample frame provided by the respective rural district assemblies and agricultural offices in the districts and villages. Table 1 detailed information on the sample

size and sampled districts and communities. Of the selected households, 249 representing 83.3% reared one or more sheep and goat livestock. Hence, such homes were considered for analysis.

Data analysis

The data were analyzed using both SPSS 16.0 and Stata version 12.0. Descriptive statistics such as frequencies and means were used to describe the data. In addition, inferential statistics including independent sample T-test (comparison of means for continuous variables), test of proportions (comparison of frequencies for discrete variables), Mann-Whitney test (comparison of means for ordinal variables) and logistic regression were used to present and explain the data.

The logistic model was employed to determine predictors for small ruminant farmer's decision to participate in veterinary services. Participation in veterinary service was the dependent variable depicting, whether or not a farmer participates in veterinary services. Farmers' participation in veterinary service was measured on a 4-point Likert-type scale ranging from 1=not accessible, 2=neutral, 3=accessible and 4=very accessible on five veterinary service activities. These activities include participation in vaccinations, advice on animal health care, treatment of diseases, sales of drugs and vaccines as well as castration of animals. An index was calculated from the 4-point Likert-type to determine a dichotomous value for veterinary service participation (that is, whether or not a farmer participates in veterinary services). Based on the 4-point scale, each farmer's index over all the veterinary service activities is calculated. For example, if a farmer's ratings for the above five veterinary service activities are 3, 4, 3, 1, and 1, then such farmer scores 2.4 (that is, (3+4+3+1+1)/5=2.4). Farmers with ratings below 2.5 (that is, (1+2+3+4)/4=2.5) were deemed not to patronize veterinary services and were coded 0. On the other hand, averages closer to 4 or from 2.5 implies those farmers participate in veterinary services and were coded 1. The explanatory variables adopted for the study were based on previous studies (Legesse et al., 2013; Meena, 2013; Okereke, 2012; Onono et al., 2013). Table 2 shows definition of each proposed independent variable that influences veterinary service participation among subsistent small ruminant farmers.

Theoretical model

Random utility maximization

The random utility (RU) function is an ideal theoretical framework to analyze economic agent's choice behaviors (Greene, 2003; Lancaster, 1966). The key assumption of the model (RU) is that economic agents (e.g., farmers) when confronted with a choice (e.g., whether or not to participate in veterinary service), will have preference of one alternative over the other (Greene, 2003; Ouma et al., 2003). Such an agent (or farmer) chooses the alternative with a higher utility over other(s). Assume that Y_j and Y_k are farmers' utility for two alternatives, represented by U_j and U_k , respectively.

The probability that a farmer will choose or decide to engage in veterinary service is that the probability of his/her utility with the new change (alternative = 1) or veterinary service participation is greater over his/her utility without the change (alternative = 0) or without veterinary service participation. In this study, the assumption is that household heads control productive assets in the house and such heads are the key decision-makers which are consistent with existing traditions in Northern Ghana. Mathematically, the household's utility is represented as follows (Ouma et al., 2003):

Table 1. Agro-ecological zones, regions, selected districts, communities, and sample sizes for the study region.

Agro-ecological zone	Region	Selected districts	Selected communities	Sample size
Guinea savannah	Northern	West Mamprusi	Kpasenkpe and Nayoku	174
		Tamale	Vitting and Kamina Barracks	
		Tolon Kumbugu	Tolon and Chirifoyili	
Sudan savannah	Upper west	Wa Municipal	Kpongu and Kolpong	72
		Nadowli	Sankana and Takpo	
		Sissala West	Siybele and Tiiwi	
Total	Upper east	Bolgatanga	Zuarungu Dachio and Sherigu Dorungu-Agobgabis	54
		Bongo	Adaboya and Gowire-Tingre	
		Bawku	Aneigo and Yarigu	
Total				300

Table 2. Definition of independent variables in the logistic regression model.

Variable	Description	Type
(a) Discrete		
Gender of household head		
Male 1	1= If household head is male	Dummy
Female 0	0= If household head is female	
Education: family-head's formal education		
Has no formal education	1 ^a = Has no formal education	Ordinal
Completed primary/JHS/SHS	2= Completed primary/JHS/SHS	
Completed college/university	3= Completed college/university	
Annual income: income of household heads in the past one year		
Less than Gh¢1,000	1 ^a = Less than Gh¢1,000	Ordinal
Gh¢1,001-5,000	2= Gh¢1,001-5,000	
Gh¢5,001-10,000	3= Gh¢5,001-10,000	
Above Gh¢10,001	4= Above Gh¢10,001	
Herd size: number of sheep and goat holdings		
Small herd size (less than 10)	1 ^a = Small herd size (less than 10)	Ordinal
Medium herd size (10 to 30)	2= Medium herd size (10 to 30)	
Large herd size (above 30)	3= Large herd size (above 30)	
Veterinary affordability: ratings of veterinary service affordability		
Not affordable	1= Not affordable	Categorical
Neutral	2= Neutral	
Affordable	3= Affordable	
Very affordable	4= Very affordable	
(b) Continuous		
Age of household heads	-	Continuous
Household size	-	Continuous

^aRefers to base category or omitted category in the analysis. JHS: Junior High Senior; SHS: Senior High School.

$$P(j) = P(I_j^* = 1) \tag{1}$$

$$P(U_j + \varepsilon_j > U_k + \varepsilon_k, k \neq j) \tag{2}$$

$$P(U_j - U_k + \varepsilon_j - \varepsilon_k > 0/x, k \neq j) \tag{3}$$

where P is the probability function, and U_j and U_k represent utilities associated with choices in option (j) and without option (k), respectively. In addition, ε_j and ε_k are the random disturbance terms. The utility theory has been applied in numerous studies to study dichotomous choice or participation in decision considerations (Deressa et al., 2008; Duku et al., 2011).

Empirical model specification

In this study, the logistic regression model was used to examine factors that influence farmers’ discrete choice of whether or not to participate in veterinary services. Of the 249 small ruminant farmers, 52% participated in veterinary services while 48% did not participate in the service. From the logistic model, the probability that a small ruminant farmer partakes in veterinary service $P(Y = 1)$ is represented as:

$$\log\left(\frac{P_j}{1-P_j}\right) = \beta_0 + \sum_{j=i}^k \beta_j X_{ij} \tag{4}$$

where $\log\left(\frac{P_j}{1-P_j}\right)$ is the log-odd ratio, X_{ij} is a vector of independent variables including personal characteristics (age, sex, educational background and household size), economic (household annual income) and farm-related (herd size, affordability of veterinary service and agro-ecological zone) variables. β_0 is the constant parameter and β_j the vector of parameters for the independent variables. The estimated parameters show the direction and effect of the explanatory variables (Maddala, 2001; Deressa et al., 2008). However to estimate the marginal probabilities which indicate the marginal change in veterinary service participation with respect to a unit change in the explanatory variables, Equation 4 is differentiated as:

$$\frac{\partial P_i \left(\log \frac{P_i}{1-P_i} \right)}{\partial x_{ij}} = \beta_j \tag{5}$$

The effect of each explanatory variable in the logistic regression model is briefly discussed as follows.

Gender: Females, unlike male counterpart farmers face higher constraints in accessing information to increase agricultural production (Asfaw and Admassie, 2004). According to Tankga et al. (2000) and supported by Miller (2009), even though, access to livestock institutions such as extension and veterinary services are insufficient in developing countries, women compared with men are the worst affected. Hence, it can be hypothesized that male farmers will have higher probability to participate in veterinary service than

female farmers, all things being equal.

Age: The effect of farmer’s age on participation in agricultural technologies such as veterinary service is mixed. Old age is used as a proxy for farming experience and can positively influence farmers’ access to improve agricultural technologies. However, Okereke (2012) found a negative relationship between age and participation in veterinary service in Nigeria. On other hand, Meena (2013) and Onono et al. (2013) reported that other factors besides farmers’ age influence the participation in veterinary service in developing countries. Similarly, Adam and Boateng (2012) also established no association between small ruminant farmer’s age and adoption of livestock technologies. It can be hypothesized that older farmers have higher probability to participate in veterinary services than younger farmers, all things being equal.

Education: It is hypothesized that the probability of farmers’ decision to participate in veterinary service increases with the level of education of farmers. Educational background of farmers is an important determinant of agricultural technology adoption, presumably because education increases farmers’ capacity to access, analyze and utilize information essential to adopt agricultural technology such as veterinary services (Legesse et al., 2013).

Household size: Family size is synonymous with available labour for agricultural production in developing countries (Sellen, 2003). Okereke (2012) reported a positive relationship between farmers’ decision to participate in veterinary service and household size because such farmers have enough labor, especially during peak seasons to adopt technologies such as visiting veterinary offices. However, Legesse et al. (2013) argued that households with larger family membership are less likely to adopt agricultural technologies due to competition for resources. In support of this claim, Yirga (2007) said that household with larger families may choose to engage in off-farm income opportunities at the expense of agricultural production and related technology adoptions. Consequently, it is hypothesized that the probability of participation in veterinary services is lower for farmers with larger family size, all things being equal.

Household annual income: Financial wellbeing of farmers is likely to positively influence adoption of agricultural technologies such as veterinary service. Gbolagade et al. (2013) found that the cost of veterinary service is the fundamental constraint limiting subsistent farmers’ participation in veterinary services in Nigeria. The majority of farmers in sub-Sahara African countries are poor, risk averse and as such those farmers may have less access to information compared with rich farmers. Hence, it is hypothesized that the probability of participation in veterinary services is higher for farmers with higher annual household income, all things being equal.

Herd size: The effect of herd/farm size on adoption of agricultural technology, including veterinary services is positive. Studies in Nigeria (Gbolagade et al., 2013; Okereke, 2012) reported a positive relationship between farmers’ livestock size and participation in veterinary service. The findings may be associated with the fact that herd size (total number of animals) is a determinant of wealth in subsistent livelihoods (Oluwatayo and Oluwatayo, 2012). Therefore, it is hypothesized that the probability of participation in veterinary services is higher for farmers with medium or large herd size compare with farmers with small herd size, all things being equal.

Veterinary service affordability: The cost of veterinary services may negatively affect farmers’ participation in veterinary services (Turkson, 2003). This is so because, livestock, especially small ruminants are raised for subsistence needs rather than market

demand in sub-Saharan African countries (Ayalew et al., 2003). In addition, local breeds are dominant and are on smallholder basis; hence, farmers may not be motivated to participate in veterinary operations if cost of service is high. However, such farmers may be encouraged to partake in veterinary services given that the cost is more affordable. In conclusion, it is hypothesized that the probability of participation in veterinary service is higher for farmers with higher perception of veterinary service affordability, all things being equal.

Agro-ecological zone: A dummy variable for agro-ecological zone (Guinea savannah = 1 and 0 = Sudan savannah) was included in the regression model. The essence is to determine whether differences in veterinary service participation exist between farmers in Guinea Savannah and Sudan Savannah agro-ecological zones.

RESULTS AND DISCUSSION

Table 3 shows the socio-economic and farm-related characteristics of farmers used in the logistic regression model. Differences in proportions between Guinea and Sudan Savannah agro-ecological zones with socio-economic characteristics differed significantly (at least 5% level) for education, annual income, herd size and veterinary service affordability except sex of farm households.

The sex distribution of farm household heads suggests that majority were males representing 81.7% in Guinea savannah and 83.7% in Sudan savannah regions. This result implies that male household heads were dominant among small ruminant farm families and such statistics are consistent with the 80% male family-heads reported in a national survey by FAO (2012) in Ghana. In addition, the data show that more than three-quarters (78.2%) of farmers in Guinea Savannah and halve (57.5%) from the Sudan Savannah zone were uneducated. These statistics are greater than the 28.5% of illiterates reported in the 2012 population and housing census in Ghana (Ghana Statistical Service, 2012). The low literacy rate has dire consequences for agricultural production in the study area, partly because such farmers may face constraints to access and use technologies to improve productivity. Similarly, a greater proportion (63.3%) of homes in the Guinea Savannah compared with Sudan Savannah (46.5%) lives below an annual income of Gh¢1,000 (US\$526). The findings imply that there are more poor people (living below \$1 a day) in the Guinea Savannah compared with Sudan Savannah agro-ecological zone. This finding is in contrast to the poverty indexes in Ghana reported by Mackay and Ayeetey (2004). However, the study agrees with the livestock data provided by Karbo and Agyare (1997a). The authors reported that more livestock animals were concentrated in Guinea Savannah (that is, entire northern region) compared with Sudan Savannah region (that is, both Upper East and West regions). This study also suggests that a greater proportion (72.8%) of farm families in Sudan Savannah compared with Guinea Savannah regions (56.7%) reared small ruminants with a flock size less than 10 animals.

The study also suggests that both farmers in Guinea (39.2%) and Sudan (47.3%) Savannah areas agreed that the cost of veterinary service is not affordable. In a related study for 4 peri-urban regions in Southern Ghana; Turkson (2008) found out that majority (89.1%) of the farmers said the cost of veterinary service is either fairly affordable or affordable. The differences in perceptions of veterinary service affordability for Turkson's study and this survey might be explained by dissimilarities in locations and poverty indexes for the two studies. While the current study was carried out in Northern Ghana which is the most poverty stricken region in Ghana, the study by Turkson (2008) was conducted in Southern Ghana.

Even though, the mean age of farmers for the two agro-ecological zones (that is, 52.0 for Guinea and 52.6 for Sudan Savannah) is not significant at 5% level, family size for the two areas differs significantly. The reported ages are slightly higher than the 47.5 years of small ruminant farmers reported by Duku et al. (2011) in the transitional zone of Ghana. The results give an indication that sheep and goat production in Ghana is dominated by the active workforce population. On the other hand, the household sizes in the two agro-ecological zones are inconsistent with the 4.4 persons documented in a national survey (Ghana Statistical Service, 2012). Subsistent agriculture systems which use family labour are predominant in northern Ghana. Consequently, the high family size indicates available labour force for livestock herding and other farming activities (Adams and Ohene-Yankyer, 2014).

Participation and reasons for not participating in veterinary services in Northern Ghana

Table 4 shows the farmer's participation and reasons for not participating in veterinary services in Northern Ghana. The variable, participation in veterinary service, is a binary indicator variable which represents percentage of farmers who participate in veterinary services. From the surveyed sample, more than half (52%) participated in veterinary services while 48% of the farmers do not use veterinary services. The aforementioned two reasons explaining why farmers do not participate in veterinary services include: (i) veterinary services are too expensive and (ii) veterinary offices are far from farms. These constraints mirror the livelihood situations in the study area. Northern Ghana is the most poverty-stricken zone in Ghana and as a result, it is unsurprising that subsistence farmers face financial constraints related matters in participating veterinary services.

Regression analysis

Table 5 shows the factors that influence farm household decision to participate in veterinary services. The log-

Table 3. Farm households' socio-economic and farm-related attributes.

Variable	Agro-ecological zones		Z-test
	Guinea Savannah (120)	Sudan Savannah (129)	
(a) Discrete			
Gender of household			
1 = Male	81.7	83.7	0.67 ^{ns}
0 = Female	18.3	16.3	
Education			
1 ^a = Has no formal education	78.2	57.5	12.28 ^{***}
2 = Completed Primary/JHS/SHS	19.3	35.4	
3 = Completed College/University	2.5	7.1	
Annual income			
1 ^a = Less than Gh¢1,000	63.3	46.5	12.15 ^{***}
2 = Gh¢1,001-5,000	25.8	31.8	
3 = Gh¢5,001-10,000	10.0	13.2	
4 = Above Gh¢10,001	0.83	8.5	
Herd size			
1 ^a = Small herd size (less than 10)	56.7	72.8	7.26 ^{**}
2 = Medium herd size (10 to 30)	20.8	14.0	
3 = Large herd size (above 30)	22.5	13.2	
Veterinary affordability			
1 = Not affordable	39.2	47.3	9.60 ^{**}
2 = Neither/not affordable	18.3	24.0	
3 = Affordable	33.3	27.1	
4 = Very affordable	9.2	1.6	
(b) Continuous			T-test
Age	52.0	52.6	0.31 ^{ns}
Household size	12.8	10.4	-2.5 ^{**}

One cedi and ninety pesewas is equivalent to one US dollar (Gh¢1.9=US\$1) during the study period. J JHS: Junior High Senior; SHS: Senior High School. ***Significance at 1%; **Significant at 5%; *Significant at 10%.

likelihood ratio with Chi-square 131.3 is significant at 1% level. This statistic implies that the application of logistic regression model to the data is justifiable. The variables that significantly influence the decision to participate in veterinary services among subsistent small ruminant producers included sex of household head, Education 2 (completed primary/JHS/SHS), household annual income 2 (Gh¢1,001 to 5,000), annual income 3 (Gh¢5,001 to 10,000), family size, herd size 2 (Medium herd size, 10 to 30 animals) and veterinary service affordability. Other factors, including the age of household head, education 3 (completed college/university), annual income 4 (above Gh¢10,001) and herd size 3 (large herd size, above 30 animals) did not significantly influence household decision to participate in veterinary services. In addition, the family size was dropped from the final model. This family size. A correlation matrix was conducted and the

results appear to suggest that households with large family size are associated with large livestock size. Hence, estimating the model with both variables will produce unreliable results.

Effect of personal factors

Sex: The logistic regression supports the hypothesis that male farmers have higher probability to participate in veterinary service than female farmers. Such probability of veterinary participation increase by 8.3%, all other factors held constant. In support of this finding, Adesope et al. (2006) found a significant and positive relationship was due to high multi-collinearity between herd size and between farmers' sex and participation in veterinary services in Nigeria. The results imply that females

Table 4. Participation and reasons for not participating in veterinary services.

Variable	Mean	Standard deviation
Participation in veterinary services ¹	0.52	0.031
Reasons for not participating in veterinary services^a		
Veterinary services are not accessible	2.85	1.15
Veterinary services are not important	1.60	0.82
No transport to carry animals to veterinary station	2.54	1.02
Veterinary offices are far from farms	3.09	0.94
Veterinary services are too expensive	3.20	0.87

¹Binary variable representing percentage of farmers participating veterinary services. ^aReasons for not participating in veterinary services are measured on a 4-point likert scale: 1, Unimportant; 2, Some how important; 3, Important; 4, Very important.

Table 5. Logistic regression results of factors influencing farm households' veterinary service participation.

Variable	Coefficient (β)	RSE (β)	Z-test	Marginal probability
Sex	0.582	0.447	1.30*	0.083*
Age	-0.006	0.013	-0.43	-0.0008
Education 2	0.796	0.416	7.91*	0.115*
Education 3	-0.074	0.840	-0.09	-0.011
Income 2	0.946	0.402	2.35**	0.134**
Income 3	0.775	0.552	1.40*	0.113*
Income 4	-0.973	0.904	-1.08	-0.133
Herd size 2	0.810	0.488	1.66*	0.115*
Herd size 3	0.275	0.489	0.56	0.038
Veterinary affordability	1.822	0.232	7.85***	0.259***
Agro-ecological zone	-0.711	0.391	-1.82*	-0.101*
Constant	-3.96	1.001	-3.96***	-
Goodness of fit and model performance statistics				
Number of observations	-	244	-	-
Likelihood ratio χ^2 (dg=13)	-	122.07***	-	-
Log likelihood	-	-107.96	-	-
Pseudo R ²	-	0.361	-	-

***Significance at 1%; **Significant at 5%;*Significant at 10%.

compared with male farmers are at a disadvantage in participation in veterinary services. Similarly, Miller (2009) argued that even though women are equally important as men in managing livestock; veterinary staff including private input sellers ignore women when providing animal health products.

Age: The hypothesis that older farmers have higher probability to participate in veterinary services than

younger farmers was not supported by the data. The finding is consistent with Legesse et al. (2012) who reported no relationship between farmer's age and decision to participate in veterinary services in Ethiopia. Onono et al. (2013) also reported that other factors besides age affect farm families' decision to participate in veterinary service. On the contrary, Okereke (2012) found age to negatively influence veterinary service participation in Izzi local government area of Ebonyi

State, Nigeria. According to this finding from Nigeria, it suggests that younger farmers were more venturesome with long term planning and as such, willing to accept innovations compared with older farmers (Adam and Boateng, 2012). However, the marginal probability of age in this study indicates that the probability of participation in veterinary service delivery decreases by 0.08%, all other factors held constant.

Education: There was a positive and significant relationship between education 2 (completed primary/JHS/SHS versus no formal education) and participation in veterinary services. Thus, farmers with primary/JHS/SHS qualification have higher tendency to participate in veterinary services than farmers without any formal education. The probability of participation in veterinary services for those farmers (primary/JHS/SHS) increases by 11.5%, all other factors held constant. The finding agrees with Adesope et al. (2006) who found that the educational background of livestock farmers is crucial to veterinary service participation. Onono et al. (2013) on the other hand, reported no relationship between farmers with primary/secondary school education and decision to participate in veterinary services. In related studies, Okereke (2012) and Legesse et al. (2013) also concluded that education is not a predetermined factor to participate in veterinary services among subsistent small ruminant farmers. The studies of Okereke (2012) and Legesse et al. (2013) seem to support the finding of no relationship between farmers with higher education levels (completed college/university versus no formal education) and participation in veterinary service reported in this study. It can therefore be concluded that while the data support the hypothesis that the probability of farmers' decision to participate in veterinary service is higher for farmers with primary/JHS/SHS qualifications, such hypothesis is rejected for farmers with college/university certificates.

Annual income: Results of the study indicate that the probability to participate in veterinary service increases by 13.4 and 11.3% for households with annual incomes of Gh¢1,001 to 5,000 and Gh¢5,001 to 10,000, respectively compared with families with incomes less than Gh¢1,000. The finding is in conformity with Okereke (2012) who reported a significant relationship between farmers' annual income and participation in veterinary services. Adesope et al. (2006) also found that farmers' income level plays a significant role in veterinary service participation. Hence, the study supports the hypothesis that the probability of participation in veterinary services is higher for farmers with high annual income compared with low income farmers. On the contrary, there was no relationship between farm households with very high income level (that is, above Gh¢10,001) and veterinary service participation. In fact, the probability of participation dropped with increase in annual income above Gh¢10,001 by 13.3%, all other factors held

constant. The decreased in veterinary participation for households with income above Gh¢10,001 can be explained by the fact that, most households are likely to be engaged in non-farm opportunities/activities. As such, such farmers may have little time to participate in livestock technology adoption strategies.

Effect farm-related factors

Herd size: The result demonstrates that the probability to participate in veterinary service increases by 11.5% for farmers with medium small ruminant herd size (that is, 10 to 30). Consistent with this revelation, Meena (2013) in India reported that farmers with medium cattle holdings have the greatest probabilities to participation in veterinary services. It appears to explain that farmers with moderate livestock holdings are more motivated to adopt livestock technologies including veterinary services. Perhaps, the financial demand to meet the veterinary requirements of such animal holdings is within the reach of subsistent farmers whom the descriptive statistics show a majority lives below US\$1 per day. In support of this fact, the data indicate lack of relationship between farmers with large small ruminant holdings (above 30) and participation in veterinary services in Northern Ghana. The finding is in line with Legesse et al. (2013) who found no relationship between farm size (small ruminant holdings) and participation in veterinary services.

Veterinary service affordability: Farmer's perception with respect to veterinary service affordability has the highest increase in the probability (26%) to participate in veterinary service. Therefore, the data confirm the probability that veterinary service participation is higher for farmers with higher perception of veterinary service affordability. The finding is consistent with the observation made by Gbolagade et al. (2013) who reported that high cost of veterinary service is the most limiting factor that hinder livestock farmers from participating in veterinary services. Hence, making veterinary service more affordable is likely to influence many less resource limited farmers to participate in veterinary services.

Agro-ecological zone: The coefficient for agro-ecological zone is negative and significant at 10% significant level. This implies that farm households in Sudan Savannah region have higher likelihood of participation in veterinary services compared with farmers from Guinea Savannah zone. The average number of livestock holdings is higher for Guinea compare with Sudan Savannah agro-ecological zone. Hence, farmers in the region (Sudan Savannah zone) may be able to manage such small or medium size holdings in terms of veterinary provisions, feeds, housing, among others.

Table 6. Mean ranks of common problems associated with health management of small ruminant animals.

Variable	Agro-ecological zones		Mann-Whitney U	Kendall's W	
	Guinea Savannah	Sudan Savannah		Mean rank	Position
Diseases and pest outbreak	126.9	123.2	7509.0 ^{ns}	3.57	1
Insufficient veterinary offices	102.1	149.3	4993.5 ^{***}	2.76	2
Insufficient drugs and medicines	108.0	140.8	5701.5 ^{***}	2.66	6
Insufficient feed stuff	119.8	129.8	7116.0 ^{ns}	2.46	7
Insufficient of animal health professionals	105.9	142.8	5447.0 ^{***}	2.75	3
Veterinary services are not affordable	103.3	145.1	5139.0 ^{***}	2.67	5
Insufficient of extension agents	108.9	140.0	5808.5 ^{***}	2.71	4
Insufficient of water source	125.5	123.6	7558.5 ^{ns}	2.19	9
Poor housing	112.2	91.6	4139.0 ^{***}	2.12	8

***Significance at 1%; **Significant at 5%; *Significant at 10%. Constraints were measured on a 4-point likert scale: 1, Unimportant; 2, Somewhat important; 3, Important; 4, Very important.

Common problems associated with health management of small ruminant livestock in Northern Ghana

The mean ranks of constraints associated with small ruminant health management are shown in Table 6. Among the surveyed farm families, diseases and pest attack was ranked as the most important animal health problem affecting subsistent sheep and goat production. The mean difference of disease and pest attack for the two agro-ecological zones is not significant. This result implies that both zones recognized parasitic disease infection as the greatest threat to livestock production. Similar studies in Ghana including Turkson (2008), Turkson and Amakye-Ansah (2005) and Turkson and Naandam (2003) reported disease and pest as the paramount animal health constraint. Adesehinwa et al. (2004) in Nigeria also observed that disease and pest posed the biggest threat to small ruminant production in the tropics. The consequences of animal disease and pest menace are numerous, including high cost of production, reduction in animal holdings and birth rate of animals.

The next two major health constraints include insufficient veterinary offices (ranked 2nd) and insufficient animal health professionals (ranked 3rd). Both constraints are ranked differently for the two agro-ecological regions. According to Turkson (2003), livestock health service stations and health professionals are inadequate in Ghana due to unfavourable policies which restrain new recruitments of veterinarians and a reduction in government technical staff for livestock production. In other related study, Gbolagade et al. (2013) identified inadequate veterinary offices as the second critical problem affecting animal health delivery among poultry farmers in Delta State in Nigeria.

The least ranked problem is insufficient drinking water for animals. The mean difference for water problem is insignificant for both agro-ecological zones. However, mean rank of constraints such as inadequate drugs and medicines, veterinary services are unaffordable, poor housing and insufficient extension agents are significantly different for both agro-ecological zones except insufficient feed stuff for animals.

Conclusion

The essence of veterinary service is to eliminate or reduce the threat posed by animal diseases and pests to both livestock production and public health. To ensure efficient and quality veterinary service delivery among subsistent small ruminant farmers, the study identifies important farmer (sex, education and annual income) and farm-related (herd size and affordability of veterinary service) attributes that influence participation in veterinary services.

Hence, such socio-economic characteristics of farmers could provide guidelines for implementation of veterinary extension programs in the study area. In addition, diseases and pest menace, insufficient veterinary offices and animal health professionals were the three top constraints that affect animal health management. This implies that sustainable livestock production can only be enhanced when animal health centers and professionals are made visible in local farming communities.

Therefore, policies that provide an enabling environment for more private veterinary practice is relevant. More so, more qualified animal health professionals need to be trained to commensurate with the increasing number of livestock smallholders in the country.

Conflicts of interest

Authors have none to declare

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A close-up photograph of a horse's face, showing its eye, ear, and part of its muzzle. The horse has a reddish-brown coat. The image is used as a background for the journal cover.

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