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<table>
<thead>
<tr>
<th>Articles</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shiga toxin-producing Escherichia coli (STEC) from Farm Animals and Humans in Tropical Africa - A review</td>
<td>10-28</td>
</tr>
<tr>
<td>M. S. Adamu, I. H. Kubkomawa, A. Ahmadu and N. S. Abubakar</td>
<td></td>
</tr>
<tr>
<td>Assessment of management practices of Tswana chickens at North East District of Botswana</td>
<td>29-38</td>
</tr>
<tr>
<td>John Cassius Moreki, Kuda Nelson and Wame Boitumelo</td>
<td></td>
</tr>
<tr>
<td>Prevalence of Haemoparasites in village chickens (Gallus gallus domesticus) slaughtered at poultry markets in Maiduguri, Northeastern Nigeria</td>
<td>39-45</td>
</tr>
<tr>
<td>J. R. Lawal, A. M. Bello, S. Y. Balami, J. Dauda, K. D. Malgwi, K. U. Ezema, M. Kasim and A. A. Biu</td>
<td></td>
</tr>
<tr>
<td>Performance and ileal characteristics of finishing Broilers fed diets supplemented with prebiotics (Mannose and Lactose)</td>
<td>46-50</td>
</tr>
<tr>
<td>Oni, O. O., Idowu, O. M. O., Oso, A. O. and Ikeobi, C. O. N.</td>
<td></td>
</tr>
</tbody>
</table>
Shiga toxin-producing *Escherichia coli* (STEC) from Farm Animals and Humans in Tropical Africa - A review

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ABSTRACT: The study was designed to review the incidences and characteristics of Shiga toxin-producing *Escherichia coli* (STEC) from farm animals and humans in tropical Africa. Serotypes O157, O26, O103, O91, O45 and O111 are usually associated with public health risks, and these serotypes are most frequently isolated from food animals. The main virulent factors of STEC associated with human diseases are potent cytotoxins (shiga toxins \(stx\_1\) and \(stx\_2\) genes, which are encoded by the \(stx\_1\) and \(stx\_2\) genes. Two additional markers also play a role in the pathogenesis of Hemorrhagic colitis (HC) and Hemolytic Uremic Syndrome (HUS): an outer membrane protein (intimin), encoded by the \(eae\) gene, and enterohaemolysin, encoded by the \(ehyA\) gene. All age groups of animals and humans can be infected with STEC, but young animals and children, the elderly, and those with compromised immune systems are the most severely affected. Little is known about factors that determine susceptibility to STEC infection and the risk factors for the development of systemic complications such as HUS. The peak age incidences (infants and young children) of classical HUS is a reflection of the age incidences of STEC infection as a whole, suggesting that susceptibility to STEC-associated HUS may, like other specific infectious diseases of childhood, be due to the lack of specific immunity, possibly to ST. The increasing recognition of STEC-associated HUS in the elderly would be consistent with waning immunity in that age group. There should be sensitization and awareness regards to the incidences and distribution of STEC serogroups O157, O26, O45, O91, O103 and O111 which are associated with public health risks in livestock and humans in tropical Africa and some parts of the world.

Key words: Incidences, characteristics, *Escherichia coli*, Livestock, Humans, Tropical Africa.

INTRODUCTION

Shiga Toxin (Stx) - Producing *Escherichia coli* (STEC), also called Verocytotoxin (VT)-Producing *E. coli* (VTEC), is one of the most important groups of diarrhea causing *E. coli* that is associated with food-borne outbreaks leading to life threatening complications (Paton and Paton, 1998; Beutin et al., 2004). The incidence of STEC in camel calves diarrhea, although not studied extensively, has been reported in Eastern Sudan (Mohammed et al., 2000) and recently, Rahimi et al. (2010) reported the prevalence of STEC O157:H7 in camel carcass during processing in Iran. A restricted range of serotypes (O157, followed by O26, O103, O91, O45 and O111) are associated with public health risks and these serotypes are most frequently isolated from food or animals (Willshaw et al., 2001; Bennett and Bettelheim, 2002). Although various STEC strains have been isolated from different animals (Beutin et al., 1993; 1995; Mohammed et al., 2003; Lengacher et al., 2010; Rahimi et al., 2010), they have been shown to be more prevalent in ruminants than in other animals (Beutin et al., 1993; Caprioli et al., 1993; Beutin et al., 1995 and Islam et al., 2008). Ruminants, mainly cattle, sheep and goats, have been established as major natural reservoirs of STEC and are known to play a significant role in the epidemiology of human infection (Hussein 2007 and Islam et al., 2007). STEC belonging to the serotype O157 have been...
extensively studied and shown to be involved in many cases and outbreaks of human diseases. Most outbreaks and sporadic cases of HC and HUS have been attributed to the STEC O157 strains (Bell et al., 1994; Conedera et al., 1997; Chapman, 1997; Elder et al., 1997 and Tarr et al., 2005). However, infections caused by some non-O157 serotypes have also been frequently associated with severe illness in humans. In some geographic areas, STEC non-O157 strains are more commonly isolated from persons with diarrhea or HUS than STEC O157 strains (Giffin et al., 1991; Pradel et al., 2000 and Moses, 2005). Out of the 2988 cases of STEC non-O157 (O104:H4) outbreaks in Germany, 759 developed HUS (WHO, 2011).

The main virulent factors of STEC associated with human disease are potent cytotoxins (shiga toxins [stx] stx1 and stx2), which are encoded by the stx1 and stx2 genes. Two additional markers also play a role in the pathogenesis of HC and HUS: an outer membrane protein (intimin), encoded by the eae gene, and enterohaemolysins, encoded by the ehaY gene (Paton and Paton1998; Karmali et al., 2010). This genetic virulent characteristics is often used in epidemiological studies to correlate between strains from various sources (Askari et al., 2010). Molecular sub typing techniques used in ten of 19 human incidents in Scotland showed that STEC O157 isolates from cattle and human cases were indistinguishable (Syne, 1997). Although STEC isolated from animals have been implicated as a cause of diarrhoea and hemorrhagic colitis in human (Gyles and Fairbrother, 2004; Radostits et al., 2007; Islam, et al., 2008 and Badouei et al., 2010).

In Nigeria, studies conducted in south-western part reported the isolation of E. coli 0157:H7 and other pathogenic E. coli strains from human patients with diarrhea (Olorunshola et al., 2000; Okeke et al., 2000a and 2003b). Similarly, the isolation of non-O157 STEC and some EPEC serotypes was reported from feces of diarrheic calves collected from various farms in Zaria, North-central Nigeria (Tekdek et al., 1995). Also in North-Eastern Nigeria, Moses, (2005) isolated STEC O157 from HIV infected patients and non-O157 from human and cattle feces. The prevalence and distribution of STEC serogroups O157, O26, O45, O91, O103 and O111 which are associated with public health risks are not popular in livestock and humans in some parts of tropical Africa in spite of the danger to public health safety. It is against this background that this study was conceived to review incidences and characteristics of Shiga toxin-producing Escherichia coli (STEC) from farm animals and humans in tropical Africa.

**Pathogenic E. coli**

Pathogenic forms of E. coli can cause a variety of diarrheal diseases in hosts due to the presence of specific colonisation factors, virulent factors and pathogenicity associated genes which are generally not present in other E. coli. Of the strains that cause diarrheal diseases, six pathotypes are now recognized. These pathotypes are: Enterotoxigenic E. coli (ETEC), Enteroinvasive E. coli (IEEC), Enteropathogenic E. coli (EPEC), Enterogregarious E. coli (EAggEC), Diffusely adherent E. coli (DAEC) and Verocytotoxigenic E. coli (VTEC) also called Shiga Toxin (Stx)- Producing Escherichia coli (STEC) (PEN, 2006).

The pioneering work that led to the discovery of the E. coli VTs was done by Konowalchuk and his colleagues during the late 1970s in Canada (Konowalchuk et al., 1977, 1978a and 1978b). While investigating the usefulness of Vero (African green monkey kidney) cells for detecting the heat-labile enterotoxin (LT) of E. coli, they observed that culture filtrates from some E. coli strains produced a profound irreversible cytopathic effect in Vero cells in contrast to the reversible cytotoxic effect of LT. Culture filtrates from 10 of 136 E. coli strains from diverse sources produced a VT effect; Seven of the strains (serotypes 026, 0128: B12, 0111:B4, 018:B21, and 0126:B16) were from infants with diarrhea, One isolate (serotype 0138:K81) was from a weanling pig, and Two VT cultures (serotypes 068:H12 and 026:K60) originated from cheese. These observations led Konowalchuk and his colleagues to speculate that VT may contribute to diarrheal disease. It should be noted that the "enterotoxic" activity of what later became known as VT had earlier been demonstrated in 1971 by Smith and Lingood (Smith and Lingood, 1971). Hardas et al. (1982) in India found that 8 of 102 E. coli strains from patients with diarrhea were VT+. These studies, however, failed to shed light on either the etiological significance of VTEC in diarrheal disease or the possible pathogenic significance of VT. The major breakthroughs occurred in 1983 with the publication of studies from the United States and Canada which linked VTEC infection to two conditions of previously unknown cause, hemorrhagic colitis (Riley et al., 1983) and HUS (Karmali et al., 1983). A classic epidemiologic investigation from the Centers for Disease Control, Atlanta, (Riley et al., 1983), linked two outbreaks of hemorrhagic colitis, a hitherto poorly understood bloody diarrhea condition, with what was then considered a "rare" E. coli serotype, 0157:H7. Shortly thereafter, the American isolates of E. coli 0157:H7 were subsequently confirmed by O'Brien and colleagues to be positive for a Shiga-like cytotoxin (O'Brien et al., 1983). Earlier work by O'Brien's group (O'Brien et al. 1982) had led to the important observation that the cytotoxin (VT) from Konowalchuk's reference strain H30 was very closely related to Shiga toxin from Shigella dysenteriae type 1. Thus developed the two different nomenclatures now used for the E. coli cytotoxins VT and Shiga-like toxin (SLT). While the epidemiological studies on hemorrhagic colitis clearly established an association...
with *E. coli* 0157:H7, the potential significance of VT in this condition remained uncertain. Studies from Canada (Karmali et al., 1983 and 1985) showed a close association between VTEC infection and the classical form of HUS, which is a leading cause of acute renal failure in childhood.

Nomenclature and definitions of STEC

The terms verocytotoxins (VT) and Shiga-like toxins (SLT) are synonymous. *E. coli* strains that produce these toxins have been referred to as VT-producing *E. coli* (VTEC), SLT-producing *E. coli* (STEC), and enterohemorrhagic *E. coli* (EHEC). The term VTEC refers to all *E. coli* strains that produce VT in culture supernatants (Konowalchuk et al., 1977; 1978a and 1978b). The term EHEC refers to strains that have the same clinical, epidemiological, and pathogenic features associated with the prototype EHEC organism *E. coli* 0157:H7. Only two VTEC serotypes (0157:H7 and 026:H11) have been classified as EHEC by Levine (Levine, 1987). The original description of SLT-producing *E. coli* included three categories of strains: (i) trace-level, (ii) low-level, and (iii) high-level producers of the toxin. High-level SLT producers contained about 100- to 1,000-fold-greater amounts of toxin than the trace- and low-level producers (O'Brien et al., 1982). Furthermore, toxin was easily detectable in supernatants of high-level producer cultures, but not in culture supernatants of trace- and low-level producers. Hemorrhagic colitis and HUS have been associated only with high-level SLT producers (corresponding to VTEC), whereas the clinical relevance of trace- and low-level SLT producing strains is uncertain. Thus, use of the term SLT producing *E. coli* can be misleading unless qualified by the amount of toxin produced. Low-level SLT production has been observed in nonpathogenic strains such as *E. coli* K-12, as well as in environmental and human isolates of *Vibrio cholerae* and *Vibrio parahaemolyticus* (O'Brien et al., 1984), but there is no evidence that these groups of bacteria are associated with either HUS or hemorrhagic colitis. Shiga-like toxin quantization, using the methods described by O'Brien and others in 1982, is not practicable in clinical microbiology laboratories. On the other hand, for routine diagnostic purposes it is feasible to examine culture supernatants for cytotoxin, i.e., cultures of strains that are of known clinical relevance and that correspond to high-level, SLT-producing *E. coli* VTEC. The term VTEC also has an ease of expression, being less cumbersome than the expression high-level SLT-producing *E. coli*.

Epidemiology of STEC Diseases

Species and serotype distribution of stx producers

It has been recognized for a number of years that STEC strains causing human disease belong to a very broad range of O:H serotypes. Richardson et al. (1987) listed 32 O serogroups (approximately 60 distinct O:H types), and the list has grown considerably since then (Bonnet et al., 1998; Paton and Paton, 1998 and Karmali et al., 2010). Although not represented in the initial group of STEC isolates described by Konowalchuk, serotype O157:H7 was the first STEC type to be linked to outbreaks of HC and HUS (Karmali, 1989). In many parts of the world, STEC strains belonging to this serotype as well as O157:H2 appears to be the most common causes of human disease. However, the relative ease of isolation of this serotype on the basis of its inability to ferment sorbitol may be contributing to an over estimation of its prevalence with respect to other STEC serotypes. Other common STEC serogroups include O26, O91, O103, and O111, and in several studies, non-O157 STEC serotypes such as these have been the predominant cause of human disease (Bielaszewska et al., 1996). There have been several reports of multiple STEC serotypes being isolated from a single patient, and in such circumstances, the contribution of each type to the pathogenesis of disease is difficult to ascertain (Kappeli et al., 2011). When one of the isolated types is O157, there is a (perhaps mistaken) tendency to ignore the potential etiological significance of the other(s).

Other members of the family *Enterobacteriaceae* are known to produce stx and to cause serious gastrointestinal disease and HUS in humans. The most notable of these is *S. dysenteriae* type 1, the causative agent of bacillary dysentery, which is frequently complicated by HUS (O'Brien and Holmes, 1987). It is the principal cause of HUS in parts of Africa and Asia (Azim et al., 1997). Disease due to *S. dysenteriae* type 1 may be particularly severe, because the organism is capable of invading the colonic mucosa, and this might result in more efficient delivery of Stx to the bloodstream, as well as significant endotoxemia. *Stx2-producing Citrobacter freundii* also causes diarrhea and HUS in humans, including one outbreak in a German child care centre (Schmidt et al., 1993). Haque et al. (1996) have described the production of a Stx1-related cytotoxin by strains of *Aeromonas hydrophila* and *A. caviae*, as judged by stx1-specific PCR and neutralization of Vero cytotoxicity with Stx1 antiserum. *Enterobacter cloacae* have also been associated with transient expression of a stx2-related gene, although its role in disease is unproven (Paton and Paton, 1996).

Incidence of STEC in animals

Hussein and Bollinger (2005a) reviewed published reports in the past 3 decades and summarized the incidence of STEC in beef cattle feces and hides. In general, the incidence rates of *E. coli* O157 ranged from 0.3 to 19.7% in feedlot cattle, from 0.7 to 27.3% in cattle
on irrigated pasture, and from 0.9 to 6.9% in cattle grazing rangeland forages. These observations suggest a high potential for infection and re-infection of cattle with *E. coli* O157 during grazing of the dense vegetation on pasture. On the range, however, cattle travel in large and less-dense areas seeking edible vegetation. With regard to testing for *E. coli* O157 at slaughter, the incidence rates ranged from 0.2 to 27.8%. Worldwide, the incidence rates of non-O157 STEC ranged from 4.6 to 55.9% in feedlot cattle and from 4.7 to 44.8% in grazing cattle. With regard to testing for non-O157 STEC at slaughter, the incidence rates ranged from 2.1 to 70.1%. These observations indicate that non-O157 STEC were prevalent in all beef production systems at rates as high as 70.1%. The incidence rates, however, varied widely and could be explained by the significant impact of environmental factors, by management practices on promoting or decreasing STEC incidences, or both. Cattle hides have been identified as an important source of microbial contamination of carcasses (Ridell and Korkeala, 1993; Bell, 1997 and McEvoy et al., 2000). It has been shown that O157:H7 and non-O157:H7 STEC can be easily transferred from cattle hides to the carcass (Barkocy-Gallagher et al., 2003). Because of the role that cattle hides can play in carcass contamination with STEC at slaughter, efforts (Bacon et al., 2000; Elder et al., 2000 and Barkocy-Gallagher et al., 2003) have been devoted to evaluate its significance. Testing swab samples from cattle hides at 12 US beef processing plants in the fall revealed a 3.6% incidence rate of *E. coli* O157:H7 (Bacon et al., 2000). In this study, no attempt was made to serotype the non-O157:H7 isolates. Cattle have long been regarded as the principal reservoir of STEC strains, including those belonging to serotype O157:H7. In Ibadan, Nigeria, Olatoye, (2010) isolated *E. coli* O157:H7 serotypes from 28.4% of 250 meat sampled. Epidemiological surveys have revealed that STEC strains are also prevalent in the gastrointestinal tracts of other domestic animals, including sheep, pigs, goats, dogs, and cats (Beutin et al., 1993). The incidence of STEC in feedlot cattle had been reported to be as high as 92% (Renter et al., 2005). Estimation of the incidence of carriage of STEC is complicated by the fact that fecal shedding may be transient and is almost certainly influenced by a range of factors including diet, stress, population density, geographical region, and season (Syne et al., 1994 and Clarke et al., 1994). Serological studies have suggested that the vast majority of cattle have been exposed to STEC at some point during their lives (Pirro et al., 1995). A study conducted to examine the incidence of STEC in seven domestic animals observed that sheep, goats and cattle were the common domestic animal reservoirs of STEC (Beutin et al, 1993). Among the animals, the incidence in sheep was the highest, followed by goats and cattle. The other domestic animals such as dogs, pigs and cats showed low prevalence of STEC. Chickens were negative for STEC. The study also indicated that 60% of the isolated bacteria were human pathogens (Beutin et al, 1993). In addition, a study has found that deer was a reservoir of STEC (Keene et al., 1997). The occurrence of *E. coli* O157 in camel has rarely been reported, in a study on camel fecal samples from the United Arab Emirates, *E. coli* O157:H7 was not identified (Moore and Mc Calmon, 2002). Studies in five east African countries on fecal and serum samples from 400 camels failed to detect STEC or anti-Stx antibodies (El-Sayed et al., 2008). However, Rahimi et al. (2012) reported an incidence of 2% in camel carcass in Iran.

While many domestic animals carrying STEC are asymptomatic, certain STEC strains are capable of causing diarrhea in cattle, particularly calves (Mohammed et al., 1986). STEC strains have also been detected in cats and dogs with diarrhea (Hammermueller et al., 1995 and Abaas et al., 1989). Natural and experimental infection of calves with an O111 STEC strain results in colitis with attachment and effacement of the colonic mucosa (Moxley and Francis, 1986). Other studies involving experimental infection with O157:H7 STEC showed that both adult cattle and calves could be transiently colonized but only neonatal calves developed significant intestinal lesions, therefore, STEC infects multiple hosts.

Due to the high prevalence of STEC in food animals, STEC is a common food contaminant in foods of animal origin. Samadpour et al., (2002) tested meat, poultry and seafood samples for shiga-like toxin genes and reported that 17% of the food samples were positive for shiga toxins. Due to the occurrence of STEC in meat and its impact on public health and food safety, USDA Inspection Service (FSIS) tests ground beef for *E. coli* O26, O45, O103, O111, O121, O157 and O145 (USDA, 2012b). STEC isolates from animal sources include the important human disease-causing serotypes, as well as a number of O:H types that have yet to be associated with human infections (Karmali, 1989).

### Incidence of STEC in humans

In terms of seasonality, STEC commonly occurs in summer (Besser et al., 1999). The seasonality of the disease agrees with the pattern of shedding of the bacteria; STEC are shed more commonly in hot months than cold months (Chapman et al, 1997). Since cattle are the reservoir for STEC, studies have found an association between cattle population and the incidence of STEC infections in humans. The incidence of STEC infection in humans was higher in areas with high cattle population and in areas where manure was used for agricultural practices (Frank et al., 2008). Similarly, other studies showed that the incidence was higher in rural
areas where people have frequent contact with cattle (Michel et al., 1999). Mead and his colleagues estimated that about 110,220 cases of STEC infections occurred each year in the US (Mead et al., 1999). A study done in Nebraska also showed that 1.2% of stool samples collected from patients with gastroenteritis were positive for STEC (Fey et al., 2000). Moreover, STEC are causing frequent food borne outbreaks. STEC infections are also the leading cause of HUS in the U.S. (Neill et al., 1987). According to CDC foodborne outbreak online database, there were four confirmed STEC outbreaks in North Dakota from 1998 to 2009. Three of the outbreaks were caused by E. coli O157 while one was caused by E. coli O111 (CDC, 2012e). Therefore, STEC infections are significant public health problem in the U.S.

Susceptibility to STEC Infections

All age groups can be infected with STEC, but young children, the elderly, and those with compromised immune systems are the most severely affected. Little is known about factors that determine susceptibility to STEC infection and the risk factors for the development of systemic complications such as HUS. The peak age incidence (infants and young children) of classical HUS is a reflection of the age incidence of STEC infection as a whole, suggesting that susceptibility to STEC-associated HUS may, like other specific infectious diseases of childhood, be due to the lack of specific immunity, possibly to ST. The increasing recognition of STEC-associated HUS in the elderly would be consistent with waning immunity in that age group.

Seasonality of STEC Infections

Information currently available indicates that human STEC infections occur most commonly in the summer and fall (Borczyk and Lior, 1987). In a study by Pearce et al. (2004), the highest prevalence of E. coli O157:H7/NM was found on meat sampled in summer and fall. However, in a study of non-O157 STEC performed on sheep, the prevalence did not follow the seasonal trend previously reported for STEC O157:H7, with the highest prevalence rates (up to 26.0%) during winter and spring (Pierard, et al., 1994). Kudva et al. (1999) hypothesized that changes in diet and/or environment influenced the seasonal variation in the prevalence of STEC O157:H7. A higher prevalence rate (10.7%) of E. coli O157 was reported when cattle hides were tested in the summer at 4 Midwestern beef processing plants (Elder et al., 2000). These different prevalence rates could be explained by sampling time (i.e., fall vs. summer). Because a large number of variables (e.g., management practices, diets fed, animal factors, and methods of STEC detection) can influence STEC prevalence, comparisons among studies should be carefully evaluated.

In a study conducted by Barkocy-Gallagher et al. (2003), they tested fecal, hide, and carcass swab samples from cattle at 3 commercial beef processing plants over 1 year. The results revealed significant seasonal differences in the prevalence rates of O157:H7 and non-O157:H7 STEC at pre-harvest (i.e., feces and hides) and post-harvest (i.e., carcasses). The prevalence rates for O157:H7 and non-O157:H7 STEC, however, varied among cattle hides (60.6 and 56.6%, respectively), feces (5.9 and 19.4%, respectively), and carcasses (26.7 and 58.0%, respectively). With regard to cattle hides, prevalence of E. coli O157:H7 was highest in the spring, summer, and fall (averaging 71.5%) and lowest in winter (29.4%). Prevalence of non-O157:H7 STEC, however, was lowest in the winter, spring, and summer (averaging 49.2%) and highest in the fall (77.7%).

It is important to note that quantitative fecal shedding of STEC is considered a more important factor than prevalence in influencing the risk of human exposure with these food borne pathogens (Omisakin et al., 2003 and Ogden et al., 2004). For example, prevalence of E. coli O157 in Scottish beef cattle at slaughter was found to be greater (P < 0.05) during the cooler months (11.2%) than during the warmer months (7.5%) (Ogden et al., 2004). This was the reverse of the known seasonality of human infections with STEC (WHO, 1998). Ogden et al. (2004) reported that high shedding beef cattle (i.e., excreting > 104 cfu/g of wet feces) to shed greater concentrations of E. coli O157 in the warmer months, which may explain increased human infections at that time. Interestingly, the high shedding cattle (9% of the cattle tested) excreted the largest amount of E. coli O157 (96%) produced.

Transmission of STEC

Outbreak investigators have identified three main routes of transmission: food borne infections (often associated with consumption of contaminated undercooked minced beef and unpasteurized milk), person to person spread, and direct or indirect animal contact (Parry et al., 1998). Direct and indirect zoonotic transmission through contact with animals or their faeces has been reported in several settings (Renwick et al. 1993; Synge and Hopkins, 1994). Although ingestion of undercooked food products of animal origin seems the likely route of transmission in most cases of human infection, there is increasing evidence that STEC infection can also be acquired via person-to-person transmission. In a day-care center outbreak (1987), no common food source was identified, and the sequential movement of illness from class to class was consistent with person-to-person transmission. In a large outbreak of E. coli O157:H7 infection in a Canadian nursing home (Carter et al., 1987) affecting 55 elderly residents and 18 staff members, the epidemic curve was biphasic. The first phase, which included the vast majority of the affected residents, was consistent
with a point source infection presumed to be ingestion of a contaminated sandwich. The second phase, which involved many of the staff who cared for the sick patients, was indicative of person-to-person transmission.

**STEC Virulent Factors**

STEC is characterized by the production of one or more types of Shiga toxins (stx1 or stx2 or their variants), which inhibit the protein synthesis of host cells, leading to cell death. stx1 and stx2 are encoded by alleles in the genome of temperate, lambdoid bacteriophages that are integrated in the *E. coli* chromosome (Strockbine et al., 1986). Besides the stx gene(s), human pathogenic STEC strains often carry the eae gene, encoding the adherence factor intimin, which is an outer membrane protein (Paton and Paton, 1998). The eae gene is carried by a pathogenicity island in the chromosome called the locus of enterocyte effacement (LEE), which is required for intimate attachment to the host intestinal mucosa (Paton and Paton, 1998). Furthermore, human pathogenic STEC strains often harbor a large plasmid encoding possible additional virulence traits such as the enterohemorrhagic *E. coli* (EHEC) hemolysin (hlyEHEC) gene, which acts as a pore-forming cytolyisin on eukaryotic cells (Schmidt et al., 1995).

However, many of the STEC strains found in the gastrointestinal tracts of domestic animals (the principal source of human infections) may have a low degree of virulence in humans. These strains are less likely to produce putative accessory virulence factors such as intimin (encoded by eae) and the plasmid-encoded enterohemolysin (encoded by enterohemorrhagic *E. coli* (EHEC) hlyA) (Schmidt and Karch, 1996). Within the human disease-associated strains, those producing Shiga toxin type 2 (stx2, encoded by stx2) appear to be more commonly responsible for serious complications such as HUS than those producing only Shiga toxin type 1 (stx1, encoded by stx1) (Ostroff et al., 1989). Furthermore, STEC belonging to serogroup O157 and, to a lesser extent, serogroup O111 are responsible for the vast majority of HUS outbreaks (Minami, 1997; Paton et al., 1996 and Griffin, 1995). Paton and Paton in 1998 developed multiplex PCR assays for the simultaneous detection of (i) stx1, stx2, eae, and EHEC hlyA and (ii) portions of the rfb (O-antigen-encoding) regions of *E. coli* O111 and O157. These multiplex PCR assays developed to rapidly determine whether a patient is infected, or food is contaminated, with STEC belonging to serogroup O111 or O157 or whether the STEC produces virulence factors associated with more serious disease.

**Clinical significance of STEC**

Evidence from studies of outbreaks (Riley, et al., 1983; Carter, et al., 1987; Beutin et al., 2004; Smith-Palmer et al., 2005; Bielaszewska, 2011) and sporadic cases (Karmal et al., 1983, 1985; CDSC, 1999; Buvens et al., 2010) of VTEC infection due in most cases to serotype 0157:H7 indicates that the spectrum of illness includes asymptomatic infection, mild uncomplicated diarrhea, hemorrhagic colitis, HUS, and thrombotic thrombocytopenic purpura (TTP) (Morrison, et al., 1985), a syndrome that is closely allied to HUS. Hemorrhagic colitis, HUS, and TTP have in the past been considered as isolated entities, although it is now clear that they span the spectrum of STEC infection, and constitute the elements by which STEC disease is recognized as a distinct clinicopathologic entity.

**Hemorrhagic Colitis**

Hemorrhagic colitis ("ischemic colitis") is a distinct clinical syndrome that presents typically with abdominal cramps and watery diarrhea followed by a hemorrhagic discharge resembling lower gastrointestinal bleeding. The disease is distinguished from inflammatory colitis by lack of significant fever and absence of an inflammatory exudate in the stools (Riley, 1987; Riley et al., 1983). Riley (1987) suggested that the disease was probably first recognized in 1971, in five young adults with a condition referred to as "evanescent colitis. Subsequently, several sporadic cases of the syndrome, going by names such as ischemic colitis and reversible segmental colitis, were described in the United States, Japan, and Europe (Karmali et al., 1983), although the etiology remained unclear. In 1982, workers from the Centers for Disease Control investigated two outbreaks of hemorrhagic colitis in Michigan and Oregon; they identified 47 individuals with hemorrhagic colitis, using a case definition of severe abdominal pain, grossly bloody diarrhea, and the lack of evidence of infection by recognized enteric pathogens. Case control studies showed that the illness was associated with ingestion of hamburgers at outlets of a well-known fast-food restaurant chain. *E. coli* 0157:H7 was recovered from the stools of about half the cases but from none of healthy controls. In a large surveillance study of hemorrhagic colitis in the United States (Wells et al., 1985), 103 patients were identified as meeting the case definition of hemorrhagic colitis over a 20-month period. *E. coli* 0157:H7 was identified in 28 (36%) of 76 cases in which stools were examined.

**Hemorrhagic Uremic Syndrome (HUS)**

Hemolytic uremic syndrome (HUS) is the most worrisome complication of EHEC infections and is characterized by the triad of acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia, with a fatality rate between 2% and 7% (Haluk, 2008). The association
between STEC and HUS was based not only on the isolation of STEC from fecal cultures, but also on the demonstration of free ST in fecal filtrates and rising levels of ST-neutralizing antibodies in patients' sera. These findings, as well as the fact that STEC isolates from these patients belonged to several different serotypes, in addition to 0157:H7, emphasized the fact that ST production was probably of direct pathogenetic significance in HUS (Schmidt et al., 1999).

Hemolytic Uremic Syndrome (HUS) has been reported in a variety of clinical and epidemiological settings, and several different agents, including drugs, chemicals, toxins, and microbes, have been postulated as potential causes (ECDC, 2011). The prevailing dogma for many years has been that HUS, which is probably a multifactorial disease, being the end result of a number of different inciting events and pathogenic mechanisms. By far the most common form of the syndrome is "idiopathic" or "classical" HUS which has its highest incidence in children. Classical HUS presents typically a few days after the onset of an acute diarrheal "prodromal" illness which is often bloody and shows remarkable similarities clinicopathologically and radiologically to hemorrhagic colitis; some patients with hemorrhagic colitis have subclinical evidence of HUS (Neill et al., 1987).

HUS is a leading, and in some centers the most common, cause of acute renal failure in childhood (Orth et al., 2007). The syndrome was at one time associated with a very high case fatality rate of about 50% (Beutin et al., 2007). However, improvement in the treatment of renal failure and the attendant biochemical disturbances, largely through the use of peritoneal dialysis, has substantially improved the outlook. Modern management techniques have reduced the case fatality rate to 10% or less, although up to 30% of survivors may develop long-term residual disability in the form of chronic renal failure, hypertension, or a neurological deficit (Nataro et al., 2011). Thus, even in the modern era HUS remains a disease with a significant mortality and morbidity. In Germany recently, out of 2294 cases of EHEC infection, 798 developed HUS (including 9 fatalities): 68% of cases were in females and 88% in adults aged 20 years or older, with the highest attack rates per 100,000 population in the group aged 20–49 years (WHO, 2011).

Attempts have been made to estimate the incidence of HUS in North America. Rogers et al. (1986) estimated the average incidence of HUS in Oregon during a 4-year period (January 1979 to December 1982) to be 0.97 and 2.65 cases per 100,000 children (18 and <5 years of age respectively). In another study in Sacramento, Rogers and her colleagues estimated the yearly incidence of HUS to be 0.41 case per 100,000 children <14 years during the study period of January 1979 and June 1982. The occurrence of an epidemic of HUS in Sacramento raised the yearly incidence to 11.2 cases per 100,000 children <14 years of age. In a study in King County, Washington, during a 10-year period estimated the yearly incidence of HUS to be 1.16 and 3.02 cases per 100,000 children <15 years of age and <3 years of age, respectively (Karmali et al., 1983). In South Africa, idiopathic HUS appears to be substantially more common in white than in black children. In England, the syndrome appears to be more common in rural than in urban areas. The reasons for such regional or socio-cultural differences in prevalence are yet to be established (Rivero et al., 2010). The occurrence of outbreak of HUS lends support to a long-held view that the etiology of the syndrome is of infectious base (York et al., 2010; WHO, 2011). Many microbes have been implicated as etiologic agents in HUS, including Shigella spp, particularly, Shigella dysenteriae typeI; Salmonella Typhi; Campylobacter jejuni; Yersinia pseudotuberculosis; Streptococcus pneumoniae (Wickham et al., 2006).

Thrombotic Thrombocytopenic Purpura (TTP)

First described in 1924 (Moschcowitz, 1924), TTP closely resembles HUS in its clinicopathological features, but differs in that neurological signs and fever are more prominent in TTP and the peak age incidence is in the third decade. In a review of 271 cases of TTP, it was noted that there was a rapidly progressive course, with 75% of patients dying within 90 days, and a pentad of clinical features: fever, thrombocytopenic purpura, microangiopathic hemolytic anemia, neurological manifestations which were often remittent, and renal dysfunction (Amorosi and Ullmann, 1966). Modern management techniques, in particular, plasmapheresis, have substantially improved the outlook (Black, 1993). Most cases of TTP present without an antecedent illness, whereas a prodromal diarrheal illness is an essential feature of classical HUS. However, two cases diagnosed as TTP have been associated with E. coli O157:H7 infection. Both cases differed from the usual form of TTP in that the patients had an antecedent bloody diarrheal illness and the disease therefore resembled classical HUS.

Pathogenesis

Production of a potent STX is essential for many of the pathological features as well as the life-threatening sequelae of STEC infection. However, pathogenesis is a multistep process, involving a complex interaction between a range of bacterial and host factors. Orally ingested STEC (often in very low initial doses) must initially survive the harsh environment of the stomach and then compete with other gut microorganisms to establish intestinal colonization. STEC organisms remain in the gut, and so STX produced in the lumen must be first
absorbed by the intestinal epithelium and then translocated to the bloodstream. This permits delivery to the specific toxin receptors on target cell surfaces inducing both local and systemic effects.

Development of Diarrhea

Based largely on evidence from animal models, three possibly mechanisms have been postulated to account for the diarrhea associated with human infection. (i) Diarrhea results from the local action of ST on the intestinal mucosa. This hypothesis has been driven by a number of experimental observations including that (a) Shiga toxin, ST1, and ST2 cause fluid accumulation in rabbit ileal loops (O’Brien and Holmes, 1987; Smith and Lingood, 1971), (b) intragastric inoculation of young rabbits with STEC O157:H7 leads to diarrhea (Pai et al., 1986), and (c) mild, nonspecific, inflammatory changes may be seen in sections of rectosigmoid biopsies from adults with E. coli O157:H7-associated bloody diarrhea which resemble changes in the colon of rabbits challenged with a crude ST preparation. Keenan et al. (1986) have conducted a detailed study of the histopathological changes associated with ST (SLT) and Shiga toxin in the rabbit ileal loop. They observed that both toxins appeared to act directly and selectively on the mature columnar epithelial cells of the intestinal villus, resulting in the premature expulsion of these cells from the lateral villus wall. The non-absorptive crypt epithelium underwent a rapid proliferation and maintained the epithelial integrity. It was proposed that the mechanism of fluid accumulation in the ileal loop probably involves fluid and electrolyte malabsorption by the non-absorptive crypt cells that release the sloughed-off mature absorptive columnar cells of the villas’ tip (Keenan et al., 1986; O’Brien and Holmes, 1987). The histological features reported by Keenan and others are similar to those of Pai and his colleagues, except that the latter observed that the epithelium of the small intestine was spared the changes despite a high concentration of ST in the small bowel. The former observed the changes in the mucosa of small bowel loops. Possible explanations for these discrepancies have been discussed in detail by Riley in 1987. Mobassaleh et al. (1988) have provided evidence that the fluid response to Shiga toxin in the rabbit small bowel loop is age dependent and correlate with the appearance of the specific toxin-binding glycolipid receptor Gb3 in the microvillus membrane after 20 days of life. Pai et al. (1986) observed a diarrheagenic response to ST in 3-dayold rabbits; the sparing of the small bowel in these young animals would be consistent with the lack of a specific receptor in the small bowel mucosa. On the other hand, the selective appearance of lesions in the colon of these young animals suggests either that the receptor Gb3 appears earlier in the colonic mucosa than in the small bowel mucosa or the colonic lesions resulted from a non-Gb3-mediated action of the toxin or other components in the crude preparation used by these workers.

The categorical demonstration of Gb3 in the human intestinal mucosa would lend strong support to the hypothesis that the receptor-mediated toxin-induced fluid accumulation in rabbits is a valid model for ST-induced diarrhea in humans. (ii) Diarrhea is related to attaching and effacing adherence of VTEC to the intestinal cells. The development of diarrhea in animal models in association with characteristic destructive attaching and effacing lesions in the large bowel mucosa is strong suggestive evidence that this may be a mechanism by which STEC cause diarrhea in humans (Tzipori et al., 1987). (iii) Diarrhea results from the systemic effects of the toxin on the intestinal vasculature. A strong case has been made that, irrespective of a possible local mucosal diarrheagenic action, ST is probably responsible for the systemic effects of STEC-associated diseases, such as HUS, resulting from an action on the microvasculature of the bowel, kidneys, and other organs and tissues. It is possible that severe toxemia results in overt hemorrhagic colitis, whereas a mild toxemia produces bowel edema and mild diarrhea resulting from fluid malabsorption. This hypothesis is supported by the observation that the injection of ST1 into rabbits leads to cecitis and diarrhea (Richardson et al., 1987). However, other factors may also be relevant since, in contrast to the mild diarrheagenic action of ST1, injected ST2 is associated with overt hemorrhagic cecitis in the rabbit model (Head et al., 1988).

Pathogenesis of systemic manifestations of VTEC Disease

Capillary endothelial cell damage is considered to be central to the pathogenesis of HUS (Fong et al., 1984). Ultrastructural studies of capillaries in tissues from patients with HUS revealed a characteristic swelling of endothelial cells accompanied by widening of the subendothelial space. Similar appearances in rabbits challenged parenterally with ST1 (Richardson et al., 1987) supports the hypothesis that endothelial cells are primary target sites for the toxin. Additional supporting evidence for this view is that endothelial cells are susceptible to the cytotoxic action of ST1 in vitro and moreover, contain Gb3 which has also been found in large quantities in the human kidney (Lingwood et al., 1987). The pathophysiological abnormalities in HUS include not only endothelial cell damage but also a reduction in the platelet count, an increase in plasma platelet aggregating activity and the occurrence of an abnormal factor VIII molecule in acute-phase plasma (Kaye et al., 1993).
Diagnosis

There are a number of difficulties associated with the diagnosis of STEC infection. In the early stages of infection, there may be very large numbers of STEC in feces; in many cases, the STEC constitutes more than 90% of the aerobic fecal flora (Paton and Paton, 1998). However, as disease progresses, the numbers may drop dramatically. In patients with HUS, the typical clinical signs may become apparent only a week or more following the onset of gastrointestinal symptoms, at which time the numbers of STEC may be either very small or the bacteria may have been eliminated from the gut altogether. Also, in some cases, diarrhea is no longer present and only a rectal swab is available at the time of admission to hospital, limiting the amount of specimen available for analysis. For these reasons, diagnostic tests should preferably be very sensitive and require minimal specimen volumes (Pollock et al., 2009). Also, the clinical presentation of STEC disease is sometimes confused with other conditions such as inflammatory bowel disease, appendicitis, intussusception and Clostridium difficile infection. Thus, rapid diagnosis is important to prevent unnecessary invasive and expensive surgical and investigative procedures or administration of antibiotic therapy which may be contraindicated for STEC infection (Chapman and Siddons, 1996).

Most VTEC studies have centered on the isolation of the single serotype, O157:H7, largely because the latter has a phenotypic property (sorbitol negative after 24 h) that facilitates detection in mixed flora on appropriate selective media. On the other hand, HUS and hemorrhagic colitis have now been associated with a wide array of different serotypes, even though O157:H7 continues to be the predominant one. The overall diagnostic strategy, therefore, must be directed towards detecting VTEC in general, rather than detecting a single serotype (Persson et al., 2007).

Diagnostic procedures are based on detection of the presence of STX or STX in fecal extracts or fecal cultures, and/or isolation of the STEC (or other STX-producing organisms). Since these procedures differ in complexity, speed, sensitivity, specificity and cost, diagnostic strategies must be tailored to the clinical circumstances and the resources available (Pollock et al., 2009).

ELISA direct detection of STX

During the past decade, a number of enzyme-linked immunosorbent assays (ELISAs) have been developed for the direct detection of Stx1 and Stx2 in fecal cultures. Like verocytotoxicity, these play a potentially important role in diagnosis because they can detect the presence of STEC (or other STX-producing species) regardless of serogroup. Such assays can also be used to confirm toxin production by putative STEC isolates where tissue culture facilities are unavailable (Pulz et al., 2003). Most of the published ELISA methods involve a sandwich technique with immobilized monoclonal or affinity-purified polyclonal antibodies to the toxins as capture ligands. After incubation with cultures, bound toxin is detected with a second STX-specific antibody followed by an appropriate anti-lg–enzyme (usually alkaline phosphatase) conjugate (Downes et al., 1989). Tests of pure isolates show that the specificities of the various STX ELISAs are in close agreement with the results of verocytotoxicity assays (Ashkenazi and Cleary, 1990). Also, Law et al. (1994) reported a specificity of 99.7% when fecal cultures were tested by STX ELISA and the results were compared with isolation of STEC. Also false-positive reactions with several strains of Pseudomonas aeruginosa using the Premier EHEC kit were reported. This included one ATCC strain which had previously been tested as negative by the manufacturer (Beutin et al., 1996). The sensitivity of the various ELISAs is affected by a number of variables including the avidity of the antibodies used and the type and amount of STX produced by a given strain. ELISAs are generally less sensitive than the verocytotoxicity assay. Downes et al. (1989) concluded that ELISA sensitivity was inadequate to reliably detect low levels of STX found in direct fecal extracts. However, the amount of free STX present in primary fecal cultures is generally greater, particularly when broths are supplemented with polymyxin B and mitomycin to enhance the release of Stx1 and Stx2 respectively. Under such circumstances, ELISAs were reported to be capable of detecting the presence of Stx1-producing organisms comprising less than 1% of total flora and Stx2-producing organisms in less than 0.1% (Law et al., 1992). Thus, under optimal conditions, STX ELISAs can provide a reliable primary screen for the presence of STEC strains (including non-O157 strains) in fecal cultures as long as the specimen is obtained fairly early in the course of infection. Studies of comparative specificity and sensitivity carried out to date indicate that commercially available stx ELISA kits are likely to be of considerable utility for laboratories without access to more specialized diagnostic procedures, particularly for detection of non-O157 STEC strains (Bennett and Tarr, 2009). However, reports of false-positive ELISA reactions indicate that independent confirmation of stx production or the presence of stx genes would be prudent. A reverse passive agglutination test for the detection of stx production is also commercially available (Oxoid, Unipath Ltd., Basingstoke, United Kingdom). The test involves incubating serially diluted polymyxin B extracts of putative STEC cultures with Stx1- and Stx2-specific antibody-coated latex particles and observing for agglutination after 24 h.
DETECTION OF STX GENES

Hybridization with DNA and oligonucleotide probes

The availability of cloned stx1 and stx2 genes enabled the development of DNA probes for the detection of STEC (Newland and Neill, 1988). Initially, probes labelled with 32P or 35S were used for testing large numbers of fecal E. coli isolates or the direct screening of colonies on primary isolation plates for the presence of stx genes by colony hybridization. These procedures were both highly sensitive and specific, and when stringent washing conditions were used, strains carrying stx1, stx2, or both could be differentiated. However, radioactively labelled probes had disadvantages for clinical laboratories, such as delays due to the need for long autoradiographic exposures, short probe half-life and the problems associated with handling and disposal of radioisotopes. These problems have been largely overcome by the introduction of nonradioactive labels such as digoxigenin and biotin, and stx probes that use these have been used for detection of STECs without loss of sensitivity or specificity (Thomas et al., 1991). The availability of nucleotide sequence data for stx genes has also permitted the design of synthetic oligonucleotide probes for detection of STEC (Karch et al., 1996). Some oligonucleotide probes were based on sequences which are highly conserved among the various toxin genes and hence permitted detection of all types. Other probes were directed against less highly conserved regions, which, under the appropriate hybridization and washing conditions, distinguished between stx1, stx2 and stx2e genes (Brown et al., 1989). Although hybridization with DNA or oligonucleotide probes is not a particularly sensitive means of screening broth cultures or fecal extracts for the presence of STEC, it is a powerful tool for distinguishing colonies containing STX genes from commensal organisms.

Polymerase Chain Reaction (PCR)

Access to sequence data for the various stx genes has also permitted the design of a variety of oligonucleotide primer sets for amplification of stx genes by PCR. Crude lysates or DNA extracts from single colonies, mixed broth cultures, colony sweeps, or even direct extracts of feces or foods can be used as templates for PCR. Stx-specific PCR products are usually detected by ethidium bromide staining after separation of the reaction mix by agarose gel electrophoresis. To date, some stxPCR assays have combined different primer pairs for stx1 and stx2, and in some cases stx2 variants, in the same reaction, thereby directing the amplification of fragments which differ in size for each gene type (Paton and Paton, 1998).

Other stxPCR assays uses a single pair of primers based on consensus sequences. These primers are capable of amplifying all stx genes with subsequent identification of the gene type requiring Southern or dot-blot hybridization with labelled oligonucleotides directed against type-specific sequences within the amplified fragment (Guion et al., 2008). Apart from increasing the sensitivity, secondary hybridization steps act as independent confirmation of the identity of the amplified product. Restriction fragment length polymorphism analysis of amplified portions of stx2 genes has also been used to discriminate between stx2 and stx2 variants (Bennett and Tarr, 2009).

In addition, PCR can be used for preparation of labelled DNA probes for use in hybridization reactions by amplification in the presence of, for example, digoxigenin-labelled nucleotides (Guion, et al., 2008). The use of PCR technology permits the detection of stx genes from samples which are microbiologically complex (such as feces or foodstuffs), including samples containing nonviable organisms. PCR assays are potentially extremely sensitive; using serially diluted DNA extracted from an STEC isolate, Brain et al. (1992) showed that amplification of less than 1,000 genomes resulted in visible stx1 and stx2 PCR products after ethidium bromide staining of agarose gels. When secondary Southern hybridization with a labelled probe was used to detect the PCR products, fewer than 10 STX-containing bacterial genomes per assay could be detected. In this study, the sensitivity was about 100-fold lower when the DNA template was prepared by direct extraction from feces seeded with known numbers of STEC. This was a consequence of the presence of inhibitors of Taq polymerase in the sample which necessitated dilution before assay. Other studies have also shown suboptimal sensitivity when PCR is carried out directly on fecal extracts (Ramotar et al., 1994). Inhibitors of Taq polymerase are also present in meat. Begum and Jackson (1995) showed that ground beef homogenates had to be diluted 1,000-fold before assay. For both feces and food samples, the sensitivity of PCR assays is vastly increased if template DNA is extracted from broth cultures (Begum and Jackson, 1995). Broth enrichment (which can involve as little as 4 h of incubation) serves two purposes; inhibitors in the sample are diluted and bacterial growth increases the number of copies of the target sequence.

PCR for detection of other STEC markers

PCR has also been used for the detection of genes encoding accessory virulence factors, such as eaeA and EHEC-hlyA, in STEC isolates (Schmidt et al., 1995). This information may be of significance, because there is a link between the presence of these genes and the capacity of an STEC isolate to cause serious human
disease (Schmidt et al., 1995). Thus, a child presenting with acute diarrhea who is infected with a STEC isolate that is also positive for eaeA and EHEC-hlyA is likely to be at increased risk of developing complications such as HUS. Fratamico et al. (1995) combined previously described STX-specific and eaeA-specific PCR primer pairs with those specific for a portion of the 60-MDa virulence plasmid from an O157:H7 STEC in a multiplex assay. They concluded that this assay was suitable for the identification of STEC strains belonging to serogroup O157. However, the O157 virulence plasmid primers actually recognize a portion of the EHEC-hlyA gene, which is not confined to serogroup O157. Thus, this particular multiplex PCR will also be capable of identifying a significant proportion of potentially virulent non-O157 STEC strains. Collectively, these assays could distinguish between EPEC and STEC strains, identify STEC strains that were likely to be of increased virulence, and identify those likely to belong to serotype O157:H7 (Gannon et al., 1997).

**Isolation of stx-Producing Bacteria**

Although a substantial amount of information on the causative STEC strain can be obtained by molecular analysis of mixed cultures, isolation of the STEC strain must be considered the definitive diagnostic procedure. Apart from confirming the molecular data, isolation permits additional characterization of STEC by a variety of methods, including O:H serotyping, phage typing, restriction fragment length polymorphism, pulsed-field gel electrophoresis and amplification based DNA typing. While this characterization may have limited clinical application, it is of great importance from an epidemiological point of view, particularly in an outbreak setting.

**Culture and immunological methods for O157 STEC**

For many years, sorbitol-MacConkey agar culture (SMAC) has been the most commonly used method for isolation of STEC because of the predominance of O157:H7 and O157:H2 strains as etiological agents of human disease in North America and Europe. Most of these strains are unable to ferment sorbitol, which distinguishes them from the majority of fecal E. coli belonging to other serotypes (March and Ratnam, 1986). Sorbitol- MacConkey agar plates are inoculated with the fecal specimen and examined after 18 to 24 h of incubation for the presence of colorless, sorbitol-negative colonies. Individual colonies are tested by slide or tube agglutination with O157 and H7 antisera. Several commercial latex reagents for O157 antigen and one for H7 antigen are also available and have been shown to be both accurate and sensitive compared with standard serological tests (Persson et al., 2007). It is still necessary to confirm STX production in tissue culture or ELISAs since not all O157 strains are toxin producers. The sensitivity of SMAC is limited by the capacity to recognize non-fermenting colonies against the background of other organisms on the plate; this is particularly difficult when the O157 strain forms less than 1% of the flora. However, Chapman et al. (1991) improved the isolation rate of O157 STEC by supplementing SMAC with cefixime, to inhibit Proteus spp. and rhamnose, which is fermented by most sorbitol-negative non-O157 E. coli strains (O157 strains generally do not ferment rhamnose). Zadik et al. (1993) reported a further improvement in O157 isolation rates by using SMAC supplemented with cefixime and potassium tellurite (CT-SMAC). Although screening fecal cultures on SMAC is inexpensive and involves minimal labor and equipment, it will primarily detect STEC belonging to serogroup O157. Serious STEC disease has been associated with many other serogroups (Johnson et al., 1990), although, some are sorbitol negative (Ojeda et al., 1995) while most are sorbitol positive. Thus, the efficacy of SMAC will vary in accordance with the local STEC serotype prevalent. In one study, SMAC resulted in the isolation of E. coli O157 from 80% of fecal samples which were positive for STX by direct cytotoxicity (Ritchie et al., 1992), whereas in another study, SMAC was positive for only 30% of verocytotoxin-positive samples (Ramota et al., 1995).

**Serology**

Diagnosis of STEC-related disease can be particularly problematic when patients present late in the course of disease, because the numbers of STEC in feces may be extremely small and hence undetectable even by PCR. In such circumstances, the etiology of infection may be established by serological means. Patients with VTEC infection develop rising levels of VT-neutralizing antibodies, and this has been used to diagnose VTEC infection in patients without other evidence of infection (Karmali, 1987). Notenboom et al. (1987) reported that patients with E. coli O157:H7 infection develop rising antibody titers to the somatic O157 antigen and suggest that O157 serology would, be of diagnostic value in some settings and helpful in investigating epidemics. Extreme caution should be exercised in interpreting the results because the O157 antigen cross-reacts with Brucella abortus (Stuart and Corbel, 1982).

**Treatment and prevention of STEC infection**

The first step in the treatment of STEC is to embarked on
intensive supportive therapy to maintain homeostasis (e.g., peritoneal dialysis or hemodialysis, fluid balance and treatment of hypertension). However, the availability of rapid and sensitive methods for the diagnosis of STEC infection early in the course of disease has provided an opportunity for instituting a specific therapeutic intervention. The objectives of therapeutic strategies would be threefold: (i) to limit the severity and/or duration of gastrointestinal symptoms, (ii) to prevent life-threatening systemic complications such as HUS, and (iii) to prevent the spread of infection to close contacts (Paton and Paton, 1998).

Antibiotic therapy might be expected to satisfy all three of the above goals. However, doubts have been raised as a consequence of retrospective studies of its efficacy in preventing the progression of STEC infection from diarrhea or bloody diarrhea to HUS. Such analyses have been compounded by variations in the types of antibiotics used, the timing of commencement of therapy in relation to onset of symptoms, and the possibility that the severity of disease may have influenced the decision to implement therapy. Nevertheless, the bulk of these studies suggested either that there was no significant benefit associated with administration of antibiotics or that therapy (either during or preceding infection) actually increased the risk of developing HUS (Tar et al., 2005; Ahn et al., 2008). However, in one study, HUS patients who had been given antibiotics during the diarrheal prodrome had milder illness (Martin et al., 1990). Examination of antibiotic use in two large O157:H7 STEC outbreaks in Scotland and Japan have also produced conflicting findings. Stewart et al. (1997) found a significant association between prior antibiotic usage and subsequent development of HUS. On the other hand, Takeda et al. (1997) found that the proportion of patients who progressed from bloody diarrhea to HUS was significantly lower when antibiotics had been administered within 3 days of the onset of symptoms, compared with untreated patients or those given antibiotics later in the course of infection. Very few prospective studies have been performed but Proulx et al. (1992) found that administration of trimethoprim-sulfamethoxazole to patients infected with O157 STEC (albeit late in the course of infection) did not prevent progression to HUS. Apart from the lack of unequivocal evidence for clinical benefit, there are theoretical arguments against the use of antibiotics. First, although STX is extracellular, much of the toxin remains associated with the STEC cell surface. Thus, antibiotics which result in cell lysis might actually increase the amount of free STX in the gut lumen available for systemic absorption. Moreover, in vitro studies have shown that treatment of O157:H7 STEC with subinhibitory concentrations of antibiotics results in a significant increase (up to 50-fold) in the amount of free STX in the culture medium (Walterspiel et al., 1992). The effect was most pronounced with antibiotics such as trimethoprim- sulfamethoxazole and ciprofloxacin, which interfered with bacterial DNA synthesis and correlated with increased induction of toxin-converting bacteriophages (Wolf et al., 1997). Cordovez et al. (1992) noted a high rate of antibiotic resistance amongst STEC, and so empirical treatment with an inappropriate drug might confer a selective advantage on the STEC over other members of the gut flora and cause overgrowth. The same risk/benefit considerations are also relevant when considering whether to administer antibiotics either to asymptomatic STEC carriers to limit the spread of infection or to uninfected close contacts of patients with proven infection to prevent acquisition. Indeed, the case for prophylaxis is weakened by reports of patients becoming infected with O157:H7 STEC while undergoing therapy for an unrelated condition with an antibiotic to which the STEC was sensitive (Tarr, 1995). More extensive randomized controlled trials are required to determine whether there is a role for antibiotic prophylaxis or therapy in STEC disease. There are also sound reasons for not administering anti-motility agents to patients with STEC diarrheal disease, since these would be expected to impede the elimination of STEC from the gut and thereby extend the exposure to STX. Indeed, retrospective analyses have shown that administration of these agents to patients with O157:H7 infection extended the duration of bloody diarrhea and increased the risk of developing HUS and central nervous system lesions (Cimolai et al., 1994; Bell et al., 1997). At present, the risks or benefits of administration of other anti-diarrheal agents such as kaolin or bismuth are not known.

**Antimicrobial Resistance of STEC**

With the emergence and dissemination of antimicrobial resistance in bacteria which is well documented worldwide (Cohen, 2000), resistance to other antibiotics was detected as early as new agents were introduced for therapeutic and growth-promotant purposes (Anderson, 1968; Matthew et al., 1998). Antibiotic resistant *E. coli* has been reported for over 50 years (Adesiyun and Kaminjolo, 1992; Lambie et al., 2000).

*E. coli*, an important gastrointestinal flora, known to be capable of accepting and transferring plasmids and which under stress readily transfers those plasmids to other species, is therefore considered an important reservoir of transferable antibiotic resistance (Enumeration of Escherichia coli and the Coliform Bacteria, 2002). Studies in the UK found that, in the late 1950s, tetracycline resistance was already detectable in *E. coli* isolates from chickens and pigs fed rations containing less than 100 g tetracycline/ton (Dunlop et al., 1998a and Orden et al., 1999). Anti-microbial resistant food borne pathogens are...
acquired primarily through consumption of contaminated foods of animal origin or water (Mead et al., 1999). Food chain, especially meat, is a major source of transmission of antimicrobial-resistant organisms to humans causing both intestinal and extra-intestinal disease (Johnson et al., 2003).

The magnitude of the public health burden due to resistant foodborne pathogens is complex and is influenced by a number of variables such as antimicrobial use practices in farming, process control at slaughter, storage and distribution systems, the availability of clean water, and proper cooking and home hygiene, among others (WHO, 2000). The major concern on the public health threat of foodborne illness is infection by antimicrobial resistant strains that lead to more intractable and severe disease (Helms et al., 2002; Martin et al., 2004). This situation is further complicated by the potential of resistant bacteria to transfer their resistance determinants to resident constituents of the human microflora and other pathogenic bacteria.

Several published data on resistance in *E. coli* originating from foods were reported from isolates cultured from retail raw meat products (Meng et al., 1988; Zhao et al., 2001 and Umolu et al., 2006). Available data from USDA-FSIS indicated that 13 million kg of ground beef and 9.5 million kg of beef trimmings were contaminated with *E. coli* O157:H7 in USA between 1999 and 2002 (Sofos, 2008). Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries including Nigeria (Okeke et al., 2005 and Aibinu et al., 2007). Unhygienic butchering and floor dressing of carcasses for meat is a common practice in Nigeria resulting in carcass contamination with pathogenic microorganisms that could cause zoonotic food poisoning (Umolu et al., 2006, Ojo et al., 2009; Olatoye, 2010). Seasonal variation could affect the degree of contamination of meat from carcasses dressed on the floor since waste water runoff or flooding plays an important role in contamination of food and ground water (Gay and Hunsaker, 1993).

White et al. (2004) suggested the need for continuous research on the ecology and epidemiology of major foodborne pathogens, and surveillance of retail food (including meat) products in order to characterize and mitigate food-borne bacterial resistance. Developed countries have national surveillance programs for monitoring of bacterial susceptibility to antimicrobials among zoonotic and commensal bacteria isolated from humans and animals. However, there are no national surveillance programs on the susceptibility of such bacteria from animals and products in Nigeria. Additionally, other authors reported the need for continuous exploration of risk assessment of the use of antimicrobials in the animal husbandry with regards to the potential public health consequences (Hald et al., 2004; Phillips et al., 2004). It was therefore hypothesized that the practice of indiscriminate use of antibiotics in livestock production results in shedding of resistant foodborne bacteria and the hygiene levels of meat processing thus the contamination of the meat destined for public consumption.

In a study on antibiotic susceptibility of *Escherichia coli* O157:H7 from beef in Ibadan Municipal, Olatoye (2010) reported eight different resistance patterns and all the isolates were resistant to one or multiple antibiotics. Tetracycline resistance was the highest (91.4%) among the isolates, while 72.9% of the isolates were resistant to nitrofurantoin and Chloramphenicol, 65.7% to cefuroxime, 44.3% to cotrimoxazole, 35.7% to nalidixic acid and 11.4% to gentamicin.

**CONCLUSION AND RECOMMENDATIONS**

It is therefore concluded that, the STEC recorded by researchers in Nigeria and Africa as a whole is high enough to pose a threat to the public health. Importantly, more STEC isolates came from cattle which is the main source of animal protein consumed by humans in the country. This calls for more veterinary attention in our slaughter slabs nationwide if the public safety is anything to go by. It is also signaling a strong warning on food processing hygiene and safety packages in Nigeria. It is therefore recommended that, there should be improvement in sanitary conditions in our slaughter houses to minimize the risk of human infections by the bacteria. Further systematic research be conducted on the meat of these animals to evaluate the level of contamination by STEC.

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N.Y. abst. 212(Vii), 115.


Assessment of management practices of *Tswana* chickens at North East District of Botswana

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ABSTRACT: The objective of this study was to document management practices, economic benefits and challenges in indigenous *Tswana* chicken production in Jackalas No.2, Moroka and Tsamaya villages of North East District of Botswana. Structured questionnaire, oral interviews and field observations were used in collecting data from 50 farmers from the three villages for six successive weeks (i.e., November to December, 2015). All data generated were subjected to descriptive statistics using frequencies, percentages and means. The obtained results showed that 98% of the poultry rearers were females. Ninety-eight percent (98%) of the farmers keep chickens mainly for meat, while two percent (2%) was for egg consumption. Family chicken production was common in the age group of 21 to 50 years. All the farmers in the study area keep *Tswana* chickens on free-range and grains were provided to supplement what they picked from scavenging. Eighty percent of the farmers in Moroka provide enclosure or confinement at night followed by Jackalas No. 2 (70%) and Tsamaya (55%). Diseases and parasites contribute to losses in chicken production and 96% of the rearers used traditional remedies to treat and control diseases with *gonde* (*Aloe sp.*) being the most common plant used. In order to increase the benefits of rearing *Tswana* chickens, the farmers should be trained in general poultry husbandry management and also be encouraged to form associations to assist them in marketing chickens. The obtained results showed that *Tswana* chickens play an important role in food security at household level of the rural populace.

Keywords: *Tswana* chickens, play important role in food security at household level of the rural populace.

INTRODUCTION

 Indigenous chickens play important roles in the rural economies of the developing and underdeveloped countries (Padhi, 2016). They are highly important livestock that supply high quality animal protein and income to the rural poor and the marginalized section of the communities (Ayieko et al., 2015; Bekele et al., 2016; Padhi, 2016). They are also important for food security and improved livelihoods (Nyoni and Masika, 2012; Ayieko et al., 2015). Meat and eggs produced from indigenous chickens are tastier and preferred by most consumers compared to that of commercial breeds (CTA, 2007). Referring to the chemical composition, colour and texture of Thai indigenous and broiler chicken muscles, Wattanachant et al. (2004) found that the amino acid profile of the indigenous chicken and broiler muscles were similar while the indigenous chickens were slightly richer in glutamic acid. Glutamic acid is an important participant of brain metabolism and is also a neurotransmitter in a numerous part of the brain synapses and acts through various ionotropic or metabotropic receptors (Kanunnikova, 2012). The other roles of glutamic acid include being endogenic anticancer agent, conjugates to anticancer agents, and derivatives of glutamic acid as possible anticancer agents (Dutta et al., 2013).

 Indigenous fowl are not a particular variety but are the result of erratic crosses between local and imported stocks (Guèye, 1998). In Botswana, indigenous chickens are referred to as *Tswana* chickens. *Tswana* chickens are comparable to indigenous chickens from other developing countries in terms of growth and overall productivity. Assan (2015) observed that indigenous chickens make a significant contribution to the livelihoods of the poor and offer substantial scope for expansion to alleviate poverty, especially in women who comprise 53% of the human population of North East district (North East...
District Development Plan 6: 2003-2009). In Botswana, Aganga et al. (2000) reported that Tswana chickens serve as a source of protein to many rural households, since farmers often slaughter the chickens for home consumption. The contribution of family chickens to household food security, poverty alleviation, economic empowerment and HIV/AIDS mitigation in Botswana is scantily documented (Moreki, 2012). The objective of this study was therefore to document management practices, economic benefits and challenges in indigenous Tswana chicken production in Jackalas No.2, Moroka and Tsamaya villages of North East District of Botswana. The main beneficiaries of this study will be the resource-poor farmers through improvement in their livelihoods.

MATERIALS AND METHODS

Study area

There are 43 villages in the North East district including Tsamaya, Jackals No. 2 and Moroka (Figure 1). Tsamaya, Jackalas No. 2 and Moroka are about 30 km, 20 km and 50 km respectively north-east of the city of Francistown. In addition, these three villages are very close to Zimbabwe border; Tsamaya is about 15 km, Moroka about 10 km, while Jackalas No. 2 is about 2.5 km. It is estimated that Tsamaya, Moroka and Jackalas No. 2 have human populations of 2387, 1692 and 1222, respectively (Central Statistics Office, 2011).

The minimum and maximum temperatures in the district in winter amounted to 5 °C and 23 °C, while in summer are 17 °C and 30 °C, respectively. North East district has annual rainfall that averages between 400 mm (in the south) and 500 mm (in the north). The rain falls only from October to March, usually in thunderstorms (North East District Development Plan 6: 2003-2009). The vegetation is characterised mainly by tree savanna with Mophane trees (Colophospermum mopane) predominating. Areas inhabited by Mophane trees have poor grass cover. Patchy grass cover is found on most of the communal lands and those parts of the freehold land, which are not overgrazed (North East District Development Plan 6: 2003-2009).

The agricultural sector is an important source of food, income, employment and investment opportunities in rural areas. Therefore, agriculture is the third largest employer in the district. Subsistence farming is practiced in most parts of the district (North East District Development Plan 6: 2003-2009). The district is prone to outbreaks of Foot and Mouth Disease due to its proximity to Zimbabwe where the disease is endemic. This district shares the border with Zimbabwe in the whole eastern side. As a consequence, the Department of Veterinary Services (DVS) continues to monitor the outbreaks and maintain the cordon fence constructed to curb cross border infections. Data supplied by DVS show that the main livestock species reared are cattle (Table 1). According to the DVS report pigs are not reared in the three villages probably due to religious taboos. The Department of Animal Production (2016) showed that Livestock Management and Infrastructure Development (LIMID) Support Programme has provided funds to 20 farmers to purchase Tswana chickens in the study area (Table 2). LIMID is one of the government support programmes that promote the rearing of Tswana chickens and guinea fowl in order to alleviate poverty in the rural areas. Besides data on LIMID beneficiaries no data on family chickens was found implying that the extension service views family poultry to be less valuable compared to other livestock species.

The main crops grown across the district are sorghum, millet, groundnuts and beans mainly because they are able to withstand drought and high temperatures. Most farmers in the North East district cultivate on average 3 to 5 hectares per household and the average age of farmers that participate in farming activities ranges from 50 to 75 years (North East District Development Plan 6: 2003-2009).

Sampling technique

Multi-stage cluster sampling with proportional allocation was used to select 50 interviewees across the three villages (Tsamaya - 20; Jackalas No. 2 -15 and Moroka - 15).

Data collection

Data were collected using structured questionnaires and direct observation. The questionnaire was divided into two parts: the first part covered demographic characteristics of the respondents and the second one was on management of Tswana chickens. Questionnaires were administered by approaching the respondents personally at their homes. Questions were read to the interviewees in the local language (i.e., Tswana) and the responses recorded in English.

Data analysis

Data were analysed using the statistical functions of Microsoft Excel for descriptive analysis such as frequencies, percentages and means, while the tables and figures were used to summarise the obtained results.

RESULTS AND DISCUSSION

Demographic characteristics of respondents

The demographic characteristics of respondents in the
three villages are stated in Table 3. The majority of respondents (38%) were aged 21-35 years followed by 36-50 years (30%), indicating that family chicken production is common in the age group of 21 to 50 years. Females and males constituted 76% and 24%, respectively. The present result on the participation of women in family chickens could be attributed to the fact that poultry is easy to handle alongside the household chores compared to cattle and small stock (sheep and goats) and/or are affordable to the rural poor. In Kenya, Onyango et al. (2016) found that 65.2% of females were involved in indigenous chicken rearing compared to 34.8% for males. Previous study by Aromolaran et al. (2013) showed that people aged 21 to 40 years were more involved in small-scale layer production. Lately, young people in Botswana have developed interest in agriculture probably because of lack of employment opportunities and the existence of government support

Table 1. Populations of cattle, goats and sheep in the study area.

<table>
<thead>
<tr>
<th>Village</th>
<th>Cattle</th>
<th>Goats</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moroka</td>
<td>447</td>
<td>293</td>
<td>12</td>
</tr>
<tr>
<td>Tsamaya</td>
<td>431</td>
<td>529</td>
<td>51</td>
</tr>
<tr>
<td>Jackalas No. 2</td>
<td>692</td>
<td>608</td>
<td>132</td>
</tr>
</tbody>
</table>

Source: Department of Veterinary Services (2015).

Figure 1. Map of North East District.
programmes such as Youth Development Fund and LIMID.

The majority of the respondents were single (46%) followed by married (30%) and others (living together and widowed) (Table 3). Ninety-four percent of the respondents had attended school with the majority (60%) having secondary school education (Table 3) while the study of Aromolaran et al. (2013) reported that all respondents were literate with 77.5% of them having higher education. The high literacy rate in this study implies that respondents could easily access government assistance support programmes and adopt new technologies with relative ease compared to those that are illiterate. Similarly, Onyango et al. (2016) also reported high literacy rate (93%) of indigenous chicken farmers in Kisumu County in Kenya.

Ninety-eight percent of poultry rearers in the present study were women and children while the remainder was males. This result indicates that chickens could play an important role in the empowerment of women. Similarly, Badubi et al. (2006) found that chickens are mainly owned and cared for by women (98%).

Table 2. Beneficiaries of LIMID Support Programmes in the study area.

<table>
<thead>
<tr>
<th>Village</th>
<th>Number of beneficiaries</th>
<th>Number of chickens</th>
<th>Amount, Pula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsamaya</td>
<td>13</td>
<td>325</td>
<td>128 248.25</td>
</tr>
<tr>
<td>Jackalas No. 2</td>
<td>3</td>
<td>75</td>
<td>29 596.75</td>
</tr>
<tr>
<td>Moroka</td>
<td>4</td>
<td>100</td>
<td>39 463.40</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>500</td>
<td>197 308.40</td>
</tr>
</tbody>
</table>

Source: Department of Animal Production (2016).

Table 3. Demographic characteristics of chicken farmers in the study area.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of respondents</th>
<th>Percent response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>38</td>
<td>76</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>21-35</td>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td>36-50</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>&gt;50</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>23</td>
<td>46</td>
</tr>
<tr>
<td>Married</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Divorced</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Others</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>Secondary</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Tertiary</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>None</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

Benefits and reasons for rearing Tswana chickens

All the respondents in the three villages kept indigenous Tswana chickens which they described as readily available, cheap to obtain and also easy to manage. In addition, Tswana chickens were said to be tolerant to harsh climatic conditions, diseases and parasites compared to commercial broilers and layers. Ninety-eight percent of the respondents said they kept poultry mainly for meat and the remainder for egg consumption. Meat and eggs provided family members with proteins which contribute to a healthy living. Alders and Pym (2009) reported that eggs offer an important source of nutrition containing approximately 315 kilojoules of digestible energy. Eggs are also one of the best sources of quality protein known. In addition, eggs supply an array of vitamins such as A, B₁₂, and K (a bone-boosting nutrient that is also involved in blood coagulation) and also provide choline, a B group vitamin that plays a role in brain development and function. Furthermore, Alders (2004) reported that eggs can be stored for several days under village conditions and require very little energy or
time to cook. However, during summer months eggs have a short shelf life due to high ambient temperatures. During this period eggs may not be stored beyond seven days without refrigeration.

The slaughter of birds for family consumption appeared to influence the size of flocks and bird population in the study area. Birds were slaughtered for home consumption in order to reduce feed costs and during disease outbreaks like Newcastle disease (NCD) in order to avoid losses. The slaughter of birds for home consumption ensured the supply of high quality protein to the families which could hardly afford other protein sources such as beef, mutton and goat meat (chevon). Ninety percent of the respondents also mentioned that they slaughtered chickens to honour a guest in the family and also that chickens were given as gifts to children after doing well in their studies.

Livestock reared

It was observed that chickens (42%) were kept alongside other livestock species, with 32% of the farmers rearing goats, cattle (16%) and other livestock (donkeys, sheep, pigs and guinea fowl) which accounted for 10% (Table 4). Similarly, the study of Aganga et al. (2000) in five villages in the Gaborone agricultural region of Botswana observed that poultry were kept in conjunction with other types of livestock especially herbivores, with 80% of the families rearing goats, 50% with cattle, 40% with donkeys and 30% with sheep. The fact that chickens were the main livestock species reared in the current study suggests that chickens play important roles in the socio-cultural lives of the rural dwellers. Another reason could be that chickens are easy to manage, require cheaper resources to establish than other livestock species and require less space compared to cattle, sheep and goats. In another study in Zambia, Simainga et al. (2011) reported that the main livestock species reared in Mongu and Kalabo districts were chickens (50.7%) followed by cattle (35.4%), pigs (7.76%) and goats (6.08%). Flock size in the current study ranged from 5 to 35 birds per household compared to 15 to 20 chickens/household reported by Badubi et al. (2006) in a study carried out in six agricultural regions of Botswana (i.e., Central, Western, Southern, Francistown, Maun and Gaborone).

In these regions, cattle were preferred livestock species than chickens. This means that the respondents in the present study could afford only chicken raising due to their economic circumstances compared to large livestock. In addition, the small size of North East District (5993 km²) does not support the rearing of livestock such as cattle, sheep and goats in large numbers due to inadequacy of grazing land.

Housing

Generally, the majority of respondents in this study provided shelter to chickens. Eighty percent of the respondents in Moroka confined poultry at night followed by Jackalas No. 2 (70%) and Tsamaya (55%). In agreement with the current results, Tan (2013) observed that village poultry free ranged during the day and were provided with shelter at night to protect them from predators, thus increasing their chances of survival. On average, 15% of respondents in the present study did not provide shelter to their birds at night, indicating that birds were prone to predation, theft and were exposed to unfavourable weather conditions.

As shown in Figure 2, housing for chickens was basic and was constructed using locally available materials such as old corrugated iron sheets, thatch grass and poles. Generally, housing was of poor structural quality and hence did not last long. Moreki (2006) argued that because of the nature of the housing system, predators, particularly cats caused losses in chicks. In the present study, chicken shelters had earth floors and had roofs that were not rainproof. The fact that shelters had no concrete floors contributed to low production as eggs might be soaked in water during the rainy season leading to poor hatchability. Earth floors made disease control extremely difficult as shelters could not be easily cleaned with water and thereafter disinfected.

In this study, women and children were responsible for locking up chickens at night. This finding is in conformity with Guéye (2005) who reported that women with the help of children are the centre of rural poultry production. Ninety percent of the respondents in the current study mentioned that they hired male persons to construct poultry shelters while the remainder indicated that husbands, brothers and sometimes uncles were involved.

<table>
<thead>
<tr>
<th>Category</th>
<th>Tsamaya</th>
<th>Moroka</th>
<th>Jackalas No. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>112</td>
<td>77</td>
<td>94</td>
</tr>
<tr>
<td>Goats</td>
<td>268</td>
<td>117</td>
<td>109</td>
</tr>
<tr>
<td>Sheep</td>
<td>28</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>Donkey</td>
<td>41</td>
<td>25</td>
<td>33</td>
</tr>
<tr>
<td>Chickens</td>
<td>336</td>
<td>348</td>
<td>299</td>
</tr>
<tr>
<td>Other poultry</td>
<td>12</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. Number of livestock reared in the study area

Moreki et al. 33
Figure 2. Housing provided to Tswana chickens by the rearers in the study area.

in the construction of shelters. In agreement with this finding, Guéye (2005) observed that though women are the centre of poultry farming, it is the responsibility of men and boys to help women in the construction of poultry shelters.

Feeds and feeding

All the respondents in this study kept Tswana chickens free-ranged and provided them with supplementary feeds in the form of grains (Figure 3) which were usually broadcasted on the ground. This finding is consistent with Addisu et al. (2013) and Bekele et al. (2016) in Ethiopia. Similarly, Moreda et al. (2013) reported that 96% of farmers provided supplementary feeding to chickens and those chickens of different age groups were fed together. Moreki (2006) also reported that birds spend a large part of the day scavenging for feeds and return home late in the evening for night shelter. Feeds provided to Tswana chickens included kitchen leftovers, grains (sorghum, maize or millet) and mixed fowl feeds (a mixture of crushed maize, sorghum and sunflower seeds) (Figure 3). Grains were mostly used as supplementary feeds at Moroka and Jackalas No. 2 (47% each). Mixed fowl feeds came second with Jackalas No. 2 having 33% and Tsamaya 30%, whereas leftovers came last in all the villages. Grains were mostly used as supplementary feeds because they were readily available, especially after a successful harvest season. Due to its high cost, mixed fowl feed was given to chickens in small quantities.

Health management

Disease control

In this study, the respondents stated symptoms of diseases which were classified into respiratory problems, diarrhoea, wounds and eye infections. This indicates that the respondents did not know the names of diseases that affected their chickens. It was, however, difficult to diagnose diseases based on the symptoms presented by respondents some symptoms were common to more than one disease. Only four percent of the rearers used veterinary drugs to treat and control diseases while the majority (96%) used traditional remedies such as gonde (Aloe sp.) and potassium permanganate. A combination of these remedies was said to be more effective by the rearers. In agreement with the current finding, Moreki (1997) in a previous study in Serowe-Palapye subdistrict found that only two percent of rearers used vaccines compared to 98% that used traditional remedies. Similarly, Mwobobia et al. (2016) in Kenya found that 80% of rural farmers used traditional products compared to 57.5% for peri-urban farmers that used conventional medicines. Only six percent of the rearers in the present study vaccinated their flocks against NCD compared to 94% that used gonde and potassium permanganate. This result implies that high mortalities due to NCD outbreaks was likely to occur in the study area because of lack of vaccinations. In addition, lack of cold chain and supply of vaccines in large doses coupled with relatively high expense of vaccine could be contributing to low use of vaccines in family chicken production. This calls for the extension service to educate respondents on the importance of disease prevention through vaccinations, as well as, vaccine handling. In agreement with the present result, Bekele et al. (2016) reported that a traditional treatment (ethnoveterinary) was the major treatment used for diseases like NCD by the majority of village chicken owners in Bench Maji Zone of South Western Ethiopia. According to Mwale et al. (2005), ethno veterinary medicine (EVM) is applied in all forms of
livestock, and even to humans especially in the stallholder farming sector of Zimbabwe. In addition, no side effects in the use of various EVM practices have been reported probably explaining why most rural communities have confidence in indigenous health management practices.

Although, different traditional remedies were used to control and treat diseases, 80% of the respondents used gonde as it was said to be the most effective remedy followed by potassium permanganate (16%). Mwobobia et al. (2016) also reported Aloe vera, neem tree, pepper and goat milk to be the main traditional products used by chicken farmers. Mwale et al. (2005) attributed the wide use of Aloe sp. to their pharmacological properties and wide distribution. Previous study of Moreki et al. (2010) reported that the leaves of Aloe sp. were used in the treatment of diarrhoea, implying that Aloe sp. could be broad spectrum as diarrhoea is a common symptom in most diseases. Mwale et al. (2006) reported that A. vera leaf and juice may be used in animals internally or externally implying that it is used for disease and parasite control. Previous study of Mwale et al. (2006) demonstrated that Aloe sp. can be used to control coccidiosis, especially among the resource-poor smallholder farmers.

**Parasite control**

The study showed that 70% of the respondents said their chickens were affected by parasites, 20% said their chickens were not affected by parasites, whereas the remainder did not indicate whether chickens were or were not affected by parasites. Furthermore, 92% of the respondents identified the main parasites of family poultry to be tampsans, lice, mites and ticks while the remainder could not. These results are in agreement with Moreki et al. (2010). As is the case with diseases, parasite control in this study was predominantly by traditional remedies such as warm or cold water with washing powder, Blue Death chemical (permethrin and carbryl) and used automobile oil. Blue Death is a multi-insect powder used to kill cockroaches, ants and crickets. Similarly, Moreki et al. (2010) reported that dips, Blue Death, wood ash and chemical dusts such as Karbadust (carbryl) were mostly used across the 10 districts of Botswana. Although no respondent in this study mentioned the use of Aloe sp. as a traditional remedy against parasites, Moreki et al. (2010) observed that Aloe sp., Jeyes fluid and burning (the area where chickens usually sleep) were some of the traditional methods used against parasites. In Kenya, Okitoi et al. (2007) reported that farmers used Mexican marigold (Tegetes minuta and Tephrosia vogelia) as insect repellents in poultry houses.

**Marketing and economic returns**

The respondents sold their chickens live or processed and these were sold throughout the year. Eighty percent of the respondents said they used income from sales to buy school uniforms for children and pay their school fees. The incomes from chicken sales were also used to purchase livestock feeds during dry periods when there is little pasture. In agreement with the current result, Alders and Pym (2009) reported that income from the sale of eggs in South Asia was used to educate children and begin the process of asset accumulation. In addition,
Alders (2004) pointed out that the sale of poultry products allows investment in other livestock such as goats, cattle, expanded poultry production and other business enterprises (Alders, 2004). Similarly, Gabanakgosi et al. (2013) in Botswana mentioned that chicken proceeds were used to purchase goats. In a related study, Moreki et al. (2010) observed that family chickens can be a stepping stone to rearing small stock and cattle in resource-poor developing countries.

Buyers of chickens were mostly neighbours, friends and passers-by. Cockerels were the first to be sold and a cockerel sold for 60-65 Botswana Pula depending on its body size followed by female adults (50-60 Botswana Pula) and growers (40-50 Botswana Pula). Cockerels were sold to maintain the male to female ratio of 1:5. Only five percent of the respondents sold manure while the rest used it in their gardens. Previous study by Moreki (2001) in 15 villages of Botswana also reported a few farmers using manure to improve fertility status of the soil, indicating that most farmers do not consider manure as a resource but as waste. Eggs were mostly harvested for family consumption to avoid losses and spoilage. In Botswana, Gabanakgosi et al. (2013) reported that eggs were sold cooked or uncooked depending on the consumer’s demand, whereas in Ethiopia, chickens and eggs are sold in local and urban markets to traders or directly to consumers depending on the location of the farm dwelling (Badhaso, 2012). Compared to other seasons, egg consumption was high in summer when temperatures and humidity are high; the conditions that contribute to egg spoilage leading to lower hatchability. Moreki (2010) reported lower hatchability rates in summer and spring due to a combination of high temperatures (especially in December and January) and rainfall.

**Some major challenges in rearing Tswana chickens**

According to Table 5, diseases accounted for 66% of losses followed by predation (38%) parasites and theft (12%). In a related study, Moreki et al. (2010) found the major causes of losses in family chickens to be diseases (36.7%) followed by diseases and parasites (11.1%), predation (8.89%) and a combination of diseases, parasites and predation (8.89%). However, in the study that was carried out by Worku et al. (2012), 96.9% of the respondents identified predators to be the primary production constraints in West Amhara Region of Ethiopia.

The most prevalent disease in this study was NCD which was the major cause of chicken losses. Vaccination against NCD was rarely done mainly because the flock sizes were smaller (5 to 35 chickens per household). Moreki (2003) attributed the little use of vaccines by family poultry rearers to the availability of vaccines in large doses (500 and 1000 doses) relative to the size of family chicken flocks which are usually small, for instance, 10 to 20 birds per household.

**CONCLUSIONS**

From this study, it could be concluded that family chickens play an important role in uplifting the standard of living of the rural households across the three villages. The income from sale of chickens was used to pay school fees for children, and buy food and clothes among others. The use of traditional remedies in managing flock health is wide across the villages. The current findings suggest that family chickens play important roles in food and nutrition security of the rural households; hence increased support from government and other stakeholders would lead to greater benefits.

**RECOMMENDATION**

Further studies should be carried out to ascertain the effectiveness of traditional remedies in chicken health management with special attention being placed on *Aloes* sp. as a common remedy used by the rearers. In addition, there is need for the extension service to educate farmers in the study area on the importance of disease prevention through vaccinations as well as vaccine handling.

**ACKNOWLEDGEMENT**

The authors wish to acknowledge the interviewees for sharing their experiences in family chicken rearing.

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Prevalence of Haemoparasites in village chickens (*Gallus gallus domesticus*) slaughtered at poultry markets in Maiduguri, Northeastern Nigeria

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ABSTRACT: The present study on prevalence of haemoparasites in village chickens (*Gallus gallus domesticus*) in Maiduguri comprising the Maiduguri Metropolitan Council (MMC) and Jere district of Borno State, Nigeria was done between November, 2015 and January, 2016. A total of 200 blood samples were collected from chickens of both sexes slaughtered in poultry market/dressing slabs located in Custom market and Monday market Maiduguri and transported to the teaching and research laboratory department of Veterinary Medicine, University of Maiduguri Nigeria, for analysis. Giemsa-stained thin blood smears were prepared and screened for the presence of haemoparasites. Microscopic examination of the thin blood smears revealed that thirty four (34) of the sampled chickens were infected with at least one genus of haemoparasites, with overall prevalence of 17.0% for *Haemoproteus*, and/or *Plasmodium* spp. Result also revealed that single infection with *Haemoproteus* spp. shows higher prevalence (50.9%) than *Plasmodium* spp. (29.4%) or mixed infection with *Plasmodium* spp. + *Haemoproteus* spp. (17.6%). There was higher sex specific prevalent rate in cock (20.5%) than in Hen (11.5%). There was also a strong association between sex and presence of haemoparasite in village chicken (χ² = 3.09). However, the distribution of the haemoparasites among the sex of the host chickens was not statistically significant (P > 0.05). Similarly the likelihood of getting more haemoparasite in male (cock) than in female (hen) is less (OR = 0.73, and 95% CI = 0.344 – 1.561).

Keywords: Prevalence, Avian Haemoparasites, Village chickens, Maiduguri, Northeastern Nigeria.

INTRODUCTION

Poultry production specifically includes chickens, ducks, guinea fowl, turkey and ostrich (Opara et al., 2012). Turkey and chicken productions however make up the main component of the commercial poultry (Opara et al., 2012). Chicken is one of the most intensively reared of the domesticated poultry species and the most developed and profitable animal production enterprise. Poultry production in Africa and parts of Asia is still distinctively divided into commercialized and village enterprise subsector, each with its peculiarities (Muchadeyi et al., 2005). The domestic chickens (*Gallus domesticus*) likely had its ancestry in the red jungle fowl *Gallus gallus* that originated from Asia. It appears that people probably domesticated chickens over 4,000 years ago, after
centuries of hunting wild jungle fowl for food (Page and Daniel, 2000) Village chickens are always associated with free-range management systems in rural areas or as backyard flocks in urban and peri-urban areas of most developing countries including Nigeria (Bebora et al., 2005). The types of feed used for this group of chickens and their feeding systems are also very typical to their group and different from those used for commercial breeds in intensive commercial farms (Bebora et al., 2005). The village chickens are however very important component in the life of villagers or those living in the rural areas (Bebora et al., 2005). Village chickens contribute immensely to rural employment of youths, serves as sources of protein to family nutrition and also serve as source of petty income (sale of eggs and birds) (Kiptarus, 2005). They also form part of cultural life of the rural dwellers used for sacrifices during cultural festivals and ceremonies as well as gifts to visitors and relatives (Bebora et al., 2005). Unfortunately, the *Gallus domesticus* can easily be infested with several types of bacterial, viral, fungal and parasitic pathogen. (Soulsby, 1982). Parasitism ranks high among factors that threaten chicken production (Mapiye et al., 2008). Among the various parasitic diseases, haemoparasites infections are the most prevalent (Soulsby, 1982). These include a number of parasites which are widely distributed in developing countries and contributing significantly to the low productivity of village chickens (Poulsen et al., 2000). Haemosporidian parasites are common blood parasites of reptiles, birds, and mammals with some stages of development in both tissues and circulating blood cells of infected hosts (Archawaranon, 2005). The most commonly recorded parasites in smears of peripheral blood are unicellular eukaryotic parasites of the genera, *Haemoproteus*, *Leucocytozoon* and *Plasmodium* (Benedikt et al., 2009). These pathogens are widespread and commonly include species from the genera *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, *Fallisia* and *Trypanosoma* (Valkiūnas, 2005; Braga et al., 2011).

Avian haemoparasites are known to be pathogenic to their hosts causing high mortalities (Merino et al., 2000; Cardona et al., 2002), these blood parasites can exert important selection pressure on their hosts through effects on survival (Sol et al., 2003; Möller and Nielsen, 2007), on reproductive success (Merino et al., 2000; Sanz et al., 2001; Marzal et al., 2005; Knowles et al., 2011), on plumage colouration with important ecological and evolutionary consequences, such as changes in community structure (Sol et al., 2003; Marzal et al., 2005; Dunn et al., 2011).

Haemoproteus species have worldwide distribution, due to migration of birds, low parasite specificity, combination of high host mobility and the limited specificity in host vector choice (Valkiūnas et al., 2005). The presence of avian Haematozoa has been reported in different areas of the world such as Italy, Bolivia, Malaysia, Czechoslovakia, India, Tanzania and Pakistan (Permin et al., 2002; Gimba et al., 2014), Nigeria (Usmana et al., 2012; Karamba et al., 2012), Ghana (Poulsen et al., 2000), Zimbabwe (Permin et al., 2002), Malawi (Njunga, 2003) and South Africa (Schultz and Whittington, 2005). This study aimed to determine the prevalence and type of avian haemoparasites parasitizing village chickens (*Gallus gallus domesticus*) in Maiduguri, Northeastern Nigeria and to provide data that can be serve as references in future research on village chickens in the study area.

**MATERIALS AND METHODS**

**Description of the study area**

Maiduguri is the capital of Borno state (Figure 1). It is located in the Sahel Savanna region of north-eastern Nigeria at latitude 11°05' North and longitude 13°05' East and at about 350m above sea level. Maiduguri has mean annual rainfall and temperature of about 630mm and 32°C respectively, but temperature can go as high as 45 to 48°C in the month of March to May. It is the largest city in northeastern Nigeria. Modern Maiduguri was founded in 1907 near the old town of Maiduguri founded in 1672. The population of Maiduguri is estimated to have crossed one million by 2009. There are many ethnic groups living in the town including Kanuri, Shuwa Arab, Babur/Bura and others. The city is a rail, road, and air transportation center serving north-eastern Nigeria and parts of Niger (www.borno-state.com).

**Collection of blood samples**

Two hundred (200) blood samples were collected at alternative days from adult village chickens of both sexes that were presented for slaughter at poultry dressing slabs in Maiduguri Metropolitan poultry markets. The poultry markets/ poultry dressing slabs includes: Custom market and Monday market. Blood sample were collected immediately into heparinized (ethylene diamine tetra acetic acid) sample bottled from the severed jugular vein following slaughter. Samples bottles were appropriately labeled, stored in a cold pack and immediately transported to the teaching and research laboratory, Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri for diagnostic procedures.

**Making of thin blood film**

A smear was prepared from blood sample using a clean grease free glass slide, a drop of blood was put on the slide and spread with another glass slide that was placed and hold at an angle 40 to 45° and push forward firmly,
the blood spread along with the movement of the spreader to have head, body and tail. The blood smear was allowed to air dry for five to ten minutes and was later fixed with absolute methanol (methyl alcohol) according to the standard procedure described by Cheesbrough (2000). The fixed labeled slides were arranged in a dry plastic slide packs.

Staining of slides

After having a successful well labeled fixed blood smear that has head, body and tail. One in ten (1/10) dilution of Giemsa stain with buffer distilled water (pH 7.2) was prepared (using 9ml buffer distilled water in 1ml of the Giemsa stain). The slides were parked in coupling jar and

Figure 1. Map of Borno State showing the study area (Maiduguri metropolitan council (MMC) and Jere) with a red pointer.
Table 1. Overall prevalence of avian haemoparasites in Village chickens slaughtered at Maiduguri poultry markets/dressing slabs, semi-arid zone of Nigeria.

<table>
<thead>
<tr>
<th>Poultry Markets /dressing slabs</th>
<th>No. examined</th>
<th>No. Positive</th>
<th>Prevalence (%)</th>
<th>P value</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Custom market</td>
<td>100</td>
<td>20</td>
<td>20.0</td>
<td></td>
<td>LL 0.289 UL 1.375</td>
</tr>
<tr>
<td>Monday market</td>
<td>100</td>
<td>14</td>
<td>14.0</td>
<td></td>
<td>0.308</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>34</td>
<td>17.0</td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

Chi square value was ($\chi^2 = 4.176$) and odd ratio was 0.651.

Table 2. Prevalence of single and mixed infection of avian haemoparasites in Village chickens slaughtered at the Maiduguri poultry markets/dressing slabs, semi-arid zone of Nigeria.

<table>
<thead>
<tr>
<th>Infection</th>
<th>Genera of avian Haemoparasites encountered</th>
<th>Number of positive samples</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Infection</td>
<td>Haemoproteus spp.</td>
<td>18</td>
<td>52.9</td>
</tr>
<tr>
<td></td>
<td>Plasmodium spp</td>
<td>10</td>
<td>29.4</td>
</tr>
<tr>
<td>Mixed Infection</td>
<td>Haemoproteus spp.</td>
<td>6</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>Plasmodium spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>34</td>
<td>100%</td>
</tr>
</tbody>
</table>

Microscopic examination of stained slides

Microscopic examination of stained slide for the presence or absence of haemoparasites were done using oil immersion lens (×100). Examination for blood parasites was carried out on tail while head was used for the labeling according to the standard procedure described by Cheesbrough (2000). Parasites are identified based on the morphological changes of the RBC as previously described by Valkiūnas (2005). Positive slides were recorded, further examined by a well and more experienced microscopist from the Teaching and Research laboratory of the Department of Veterinary Medicine, University of Maiduguri.

Data analysis

Statistical analysis was performed using Chi square test with SPSS (statistical) software version 20 to test association of sex of chicken with the presence or absence of infection (variable that reflect prevalence, define as infection status: N= not infected chicken, PP= positive for Plasmodium spp, PH= positive for Haemoproteus spp and PHP= mixed infection with Haemoproteus spp + Plasmodium spp.).

RESULTS

Table 1 shows the overall prevalence of avian Haemoparasites in Village chickens slaughtered at the major poultry markets/dressing slabs namely: Custom Market and Monday Market in Maiduguri metropolitan council. Out of the two hundred (200) blood samples collected and examined microscopically for the presence or absence of avian haemoparasites, thirty four (34) were positive with an overall prevalence rate of 17.0%. The findings also show that the relative prevalence of avian haemoparasites is higher in samples collected from Custom Market (20.0%) than Monday Market (14%). Table 2 shows the result of the genera of avian haemoparasites encountered in Village chickens slaughtered at poultry markets/dressing slabs in Maiduguri. Two genera of avian haemoparasites which includes Haemoproteus spp. and Plasmodium spp. were encountered in the infected blood samples (34). Each of these parasites occurred in a single infection at a relative prevalent rate of 18 (52.9%) and 10 (29.4%) respectively. Mixed infection with Haemoproteus spp. + Plasmodium spp. were also encountered in some of the blood samples at a relative prevalent rate of 6 (17.6%). Table 3 shows the result of prevalence of avian haemoparasites in village chickens slaughtered in Maiduguri poultry markets/dressing slabs based on sex of chickens. Out of the 112 blood samples collected from Cocks and 88 from Hens, 23 (20.5%) and 11 (12.5%) chickens were found positive for different genera of avian haemoparasites respectively.

DISCUSSION

This study was conducted using the microscopy diagnostic method that is considered in most haemoparasitology as the “gold standard” diagnostic
Table 3. Prevalence of avian Haemoparasites in village chickens according to sex slaughtered at poultry Market/dressing slabs in Maiduguri, semi-arid zone of Nigeria.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of Samples examined</th>
<th>Number of Positive samples</th>
<th>Prevalence (%)</th>
<th>P value</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cock</td>
<td>112</td>
<td>23</td>
<td>20.5</td>
<td>0.420</td>
<td>LL UL</td>
</tr>
<tr>
<td>Hen</td>
<td>88</td>
<td>11</td>
<td>12.5</td>
<td>0.344</td>
<td>0.420 1.561</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>34</td>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chi square value was ($\chi^2 = 3.09$) and odd ratio was 0.73.

The result of this study revealed the prevalence of avian haemoparasites in village chickens ($Gallus gallus domesticus$) slaughtered at the major poultry markets/dressing slabs in Maiduguri; semi-arid zone of Nigeria. Out the 200 blood samples collected and microscopically observed for the presence of avian haemoparasites, 34 chickens were infected with an overall prevalence of 17%. They were infected with Haemosporidia which includes parasites from two genera namely Haemoproteus spp and Plasmodium spp. This report is consistent with that of Karamba et al. (2012) who have also reported the same genera of avian haemoparasite in domesticated and wild birds in Kano State, Nigeria. Although, the findings of this present study differs from the numbers of previously reported genera of avian haemoparasites in village chickens in Malaysia (Siong et al., 2010; Gimba et al., 2014), Iraq (Shadan, 2013; Hasson, 2015), Kenya (Sabuni et al., 2011), Zimbabwe (Permin et al., 2002) and Nigeria (Sadiq et al., 2003; Usmana et al., 2012). Most of these studies have reported the prevalence of three or more genera of avian haemoparasites in Village chickens which includes Haemoproteus species, Plasmodium species, Leucocytozoon and Trypanosoma species. The present study did not detect Leucocytozoon species, Trypanosoma species, Babesia species nor Filaria species from samples examined. The difference in the findings could be as a result of differences in habitat, climate, behaviour and diet of chickens (Siong et al., 2010). In addition, it is likely that the differences in the prevalence of avian haemoparasites infection reported are due to factors related to the methods used for diagnosis, vector/arthropod breeding season, sampling effort and location, including poultry species and the abundance of arthropod vectors responsible for transmitting the parasites (Braga et al., 2011; Gimba et al., 2014; Bell et al., 2015). The prevalence rate of 17.0% of avian haemoparasites reported in present study is in line with 19.56% that was reported by Karamba et al. (2012) but lower than 79.2% and 76.0% reported by Sabuni et al. (2011) and Hasson (2015) respectively.

The positive samples found were either infected with single or mixed infection. Single infection with either Plasmodium spp. or Haemoproteus spp. occurred at a prevalent rates of 10 (29.4%) and 18 (52.9%) respectively, Haemoproteus spp. was the most prevalent avian haemoparasite in village chickens in this present study. This finding is consistent with previous report by Permin et al. (2002); Gimba et al. (2014) and Hasson (2015) who also reported that Haemoproteus spp. is the most frequently encountered avian haemoparasites in Village chickens in their respective study area. However, the findings of this study contrast that of Sadiq et al. (2003) and Shadan (2013) who reported Plasmodium spp as the most encountered avian haemoparasites in their study areas.

Mixed infection with two haemoparasites (Plasmodium spp.+ Haemoproteus spp.) was found at a prevalent rate of 6 (17.6%). This finding also agreed with previous report by Hasson (2015). This variation can be adequately attributed to variation between agro climatic conditions and availability of arthropod vectors (Permin et al., 2002).

The result of this present study revealed that there was higher specific prevalent rate of haemoparasite in male chickens (20.5%) as compared to the female chickens (11.5%). There was also a strong statistical association between sex of birds and the presence of haemoparasite in infected village chicken ($\chi^2 = 3.09$). However, the distribution of the haemoparasites among the sex of the host chickens was not statistically significant (P > 0.05). Similarly there was no much likelihood of getting more haemoparasite in relation to sex (OR= 0.17, and 95% CI = 0.17 – 0.22). This finding is consistent with previous report by Al-Barwari and Saeed (2012) but contrast the findings of Hasson (2015) who reported high prevalence in female than male chicken. The higher prevalence of avian haemoparasites in male chickens as reported in this present study may be attributed to the abundance of predilection site for blood seeking arthropods. This is connected to the facts that male chickens anatomically have larger comb and wattle which are well supplied with blood vessels that may attract blood sucking arthropods for blood meal during which they may transmit haemoparasites to the host bird. Although, several endogenous and exogenous factors may have an accumulative influence on the parasitisation of both sexes of the village chickens by these parasites, such as host's hormones and humoral compounds, age and nutritional state, behaviour and habits, as well as the season of the year and ecological and physical features.
of the regions (Hasson, 2015). However, this present study was unable to detect or categorize the prevalence of avian haemoparasites in village chickens based on age of birds. This is because the study limited its scope to blood samples collection from adults village chickens presented for slaughter at the poultry markets/dressing slabs in the study area.

CONCLUSION

The study which is first of its kind in Maiduguri confirms the presence of avian haemoparasites genera of Haemoproteus species and Plasmodium species among adult Village chickens of both sexes presented for slaughter at poultry markets/dressing dressing slabs in Maiduguri, North-eastern Nigeria. As such, microscopic detection of avian haemoparasite gametocytes within Chicken RBC calls for attention in the study area. Despite carrying out the study during the dry season period which was considered a non-breeding season for most arthropod vectors, especially the mosquitoes, 17% prevalence rate was recorded. The infection is more among the male chickens and female chickens which revealed both sexes shared equally chances of getting infected with both detected blood parasites. The fact that this study limited its scope to investigation of parasites in adult chickens, occurrence of the parasites in young chickens should not be under estimated since both age groups mingle together at all times of the days and nights, therefore sharing equal chances of exposure to arthropod vectors that transmit the infection. Although, this call for further parasitological investigation by researchers in the study area.

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Performance and ileal characteristics of finishing Broilers fed diets supplemented with prebiotics (Mannose and Lactose)

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ABSTRACT: An experiment was conducted to evaluate the effect of prebiotics supplemented diets on performance characteristics and gut morphology of broiler chickens. The study involved 320 day-old Anak broiler chicks, used to assess the utilization of prebiotics [Mannose oligosaccharides (MOS) and Lactose oligosaccharides (LOS)] by broiler chicks. The chicks were allotted to eight treatment groups of 40 birds each and four replicates of 10 birds each in a 2 x 4 factorial arrangement. The additives (MOS and LOS) were added to the diets at four levels (0, 250, 500 and 750 ppm) per additive. The birds were fed for 56 days, after which data on feed intake, body weight gain, feed conversion ratio and ileal morphology were collected. The data was subjected to analysis of variance and significant means were separated using Duncan’s Multiple Range Test. Results showed that final weight values significantly (p<0.05) ranged from 1276.67 to 1503.38 g. The ileum morphology of the finishers broilers showed that villi height, lamina propria depth, basal width and apical width were significantly (p<0.05) influenced by prebiotic sources and levels. The villi height of 2250.00 µm was highest at 500 ppm MOS level of inclusion, while the least value of 538.30µm was obtained in birds fed 0ppm Lactose inclusion. It was therefore, concluded that, prebiotic MOS at 500ppm could be used in feed to obtain the better weight gain and FCR, with normal gut morphology of broiler chickens.

Key words: Performance, ileal morphology, broilers, mannose oligosaccharide, lactose oligosaccharide.

INTRODUCTION

Antibiotics have been used in agriculture to promote growth and welfare of animals for the past 80 years in the United States and other countries (Dibner and Richards, 2005). The long term and extensive use of antibiotics in human and veterinary medicine have resulted in selection of resistant bacterial strains. Resistance among gram-negative bacteria, like E. coli and Salmonella spp. has generated the strongest objection to antibiotic use (Gustafon and Bowen, 1997). As an illustration of many other surveys in the literature conducted to show antimicrobial resistance, Nayak and Kenney (2002) showed that 25% of the Salmonella isolates from turkey flocks in West Virginia were resistant to one or more antibiotics, including gentamycin, spectinomycin, streptomycin, tetracycline, tobramycin and trimethoprim. The European Union ban on the use of most of the sub-therapeutic antibiotics in animal feed was based on the fears of antibiotic resistance being transferred via the food chain and proposed the precautionary principle since 1997 (Cervantes, 2005). Cervantes (2005) reported that, the ban of antibiotic feed additives has resulted to significant decrease of antibiotic resistance among bacteria isolated from raw meat products. Additionally, associated with the ban on feed-additive antibiotic was a rise in the incidence of colibacillosis and necrotic enteritis in poultry (Truscott and Alsheikhly, 1997; Ferket, 2003)
and pigs (Casewell et al., 2003; Cervantes, 2005).

However, with the limitation of antibiotic growth promoters (AGPs), the consequent need for their total withdrawal becomes necessary. Hence, the need to find alternative feed supplements that have probiotic and prebiotic effects and promote growth of broiler chickens, thus achieving both enhanced performance and good health without the use of antibiotics. In order to find better alternatives to AGPs, research has focused on utilization of feed additives such as enzymes, probiotics, prebiotics, symbiotic products and even nutrient to enhance gut health and prevent or limit production losses due to enteric infections.

Prebiotics are dietary components that are not digested by the host, but they benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the gastro-intestinal tract (GIT), predominantly those that produce short chain fatty acids (SCFA). However, dietary supplementation of prebiotics has been shown to stimulate these unculturable bacteria in humans (Rastall et al., 2005), and pigs (Konstantinov et al., 2003).

Lactose is a major type of sugar found in milk and milk products, including human milk. It constitutes less than 80% of the solid in milk, in animals. Lactase and enzymes produced by the small intestine break down lactose so it can be absorbed into the blood stream. Lactose is one of the prebiotics and it functions by lowering the gut pH through lactic acid production, inhibiting/preventing colonization of pathogens, modifying metabolic activities of normal intestinal flora and stimulation of the immune system (Fons and Tuomo Karjalainen, 2000).

Mannan oligosaccharides are derived from yeast cell wall and work slightly differently from fructo-oligosaccharides. The major difference between fructo-oligosaccharides and mannan-oligosaccharides (MOS) is that MOS products do not selectively enrich for beneficial bacteria. The binding and removing of pathogens while stimulating the immune system are the primary mode of action for MOS products (Patterson and Burkholder, 2003). Therefore, this study was designed to evaluate the effects of prebiotic supplemented diets on performance and gut morphology of finishing broiler chickens.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Poultry Unit of the Directorate of the University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. It falls within the rainforest vegetation zone of South-Western Nigeria at latitude 7°13’ 49.46”N, longitude 3°26’ 11.98”E and altitude of 76 meters above sea level. The climate is humid with a mean annual rainfall of 1037 mm. The annual mean temperature and humidity is 34.7°C and 82% respectively.

Experimental diets

A basal diet was formulated to conform to the nutrient requirements of broiler chickens in the Tropics according to NRC (1994) as shown in Table 1. Diets were prepared by adding Lactose oligosaccharide or Mannan oligosaccharide at 0, 250, 500 and 750 ppm, and this gives a total of eight diets.

Experimental birds, design and management

A total of 320 day-old broiler chicks of commercial strain (Anak et al., 2000) were purchased from Nubreed Farms Ltd., Abeokuta, Ogun State, Nigeria. The birds were fed ad libitum on broiler starter for a period of 28 days (Table 1) and finisher diet (Table 2) from week 5 to 8. They were allocated randomly to eight dietary treatment groups of 40 birds each. Each treatment was further divided into four replicate groups of ten birds each. The birds were assigned randomly to eight dietary treatments in a 2 × 4 factorial arrangement. Prebiotics (Lactose and Mannose) were included in a 2 × 4 factorial arrangement of 4 inclusion levels (0, 250, 500 and 750 ppm) and two prebiotic sources (Lactose and Manose). Fresh and clean water was supplied ad libitum. All the routine and occasional management practices were carried out as at when due using standard practice.

Data collection

Data were collected on growth indices such as: weight gain, feed intake and feed conversion ratio. Feed conversion ratio (FCR) was calculated as the ratio (kg/kg) of average daily DM intake to average daily BW gain.

Gut morphology of broiler chickens

This analysis was carried out at the Veterinary Pathology Laboratory of Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. At the end of the experiment (56 days), one bird per replicate was slaughtered. The small intestine (ileum, duodenum and jejunum) was excised immediately and fixed in formalin for the measurement of villus height (VH), Apical width (AW), Lamina Propria Depth (LPD) and Basal width (BW). They were determined at a magnification of x10.

Proximate analysis

The proximate compositions of the diets were determined according to the methods described by AOAC (2000).
Table 1. Gross composition of Experimental Diets to be fed to broiler chicks (0-4weeks) (g/kg).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Lactose</th>
<th>Mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 ppm</td>
<td>250 ppm</td>
</tr>
<tr>
<td>Maize</td>
<td>460.00</td>
<td>460.00</td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>330.00</td>
<td>330.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td>30.00</td>
<td>30.00</td>
</tr>
<tr>
<td>Wheat offal</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>LOS</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>MOS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>30.00</td>
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<td>Bone meal</td>
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<td>20.00</td>
</tr>
<tr>
<td>Salt</td>
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<td>2.50</td>
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<tr>
<td>Premix</td>
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<td>Methionine</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Total</td>
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<td>1000</td>
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Calculated Analysis

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<tr>
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<td>1.78</td>
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<td>1.78</td>
<td>1.78</td>
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<td>0.45</td>
<td>0.45</td>
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<tr>
<td>Lysine (%)</td>
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<td>1.30</td>
<td>1.30</td>
<td>1.30</td>
<td>1.30</td>
<td>1.30</td>
<td>1.30</td>
</tr>
<tr>
<td>Methionine (%)</td>
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<td>0.65</td>
<td>0.65</td>
<td>0.65</td>
<td>0.65</td>
<td>0.65</td>
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</tr>
</tbody>
</table>

Vitamin and mineral premix contained the following per kg diet: Vit 4,000.000iu, Vit D 80000, Vit B12 25mg, Niacin 6000mg, Vit E 40000, Vit K3 800mg, Vit B3 1000mg, Vit B6 5000 mg panthotenic acid 20000, Follics Acid 200 mg, Biotine 8 mg, Magnanese 30000, Iron 8000 mg, Zinc 2000mg, Copper: nill, Cobalt: 80 mg, Iodine: 400 mg, Selenium: 40mg, Choline: 80000 mg. ** Lactose: 0 ppm L.S, +: 250 ppm L.S, ++: 500 ppm L.S, +++: 750 ppm L.S, ** Mannose: 0 ppm B.C, +: 250 ppm B.C, ++: 500 ppm B.C, +++: 750 ppm B.C.

Statistical analysis

All data collected were analysed using the General Linear Model of SAS (1999), and means were subjected to analysis of variance (ANOVA) in a 2 x 4 factorial design (SAS, 1999), while significant (P<0.05) means were compared using Duncan’s Multiple Range Test (Duncan, 1955).

RESULT AND DISCUSSION

The interaction effect for prebiotic sources and levels on the performance of broiler finisher is shown in Table 3. Total feed intake and FCR were affected (P<0.05) by sources of prebiotic supplemented diets. All birds fed with lactose and 500 ppm mannose had higher (P<0.05) values of final live weight and total weight gain. Improved (P<0.05) feed conversion ratio was observed on birds fed with mannose-supplemented diet at 500 ppm while other dietary treatments recorded lower values. This was in line with findings of Hooge (2004) who concluded that, birds fed with MOS showed improved growth performance and feed conversion ratio compared to birds fed diets containing antibiotic growth promoters. Also, Blake et al. (2006) indicated that, the addition of MOS to broiler diets showed a positive influence in promoting body weight gain with values far above birds fed control diet. Higher final live weight, weight gain and improved FCR recorded for broilers fed 500 ppm mannoligossaccharides (MOS) suggested the superiority of mannose over lactose (LOS) in supporting growth and feed utilization.

The result of the interaction effect of prebiotic sources and levels on the ileum morphology (Table 4), showed that villi height, lamina propria depth, basal width and apical width of broiler finishers were influenced (P<0.05) by prebiotic sources and levels. The villi height (P<0.05) was highest at 500 ppm mannose level of inclusion (2250.00 um), while it was least (583.30 um) at 750 ppm mannose inclusion. Higher (P<0.05) values in BW of birds fed 500 ppm mannose was recorded, and the least
Table 2. Gross composition of experimental diets fed to broiler finishers (4-8 weeks) (g/kg).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>0 ppm</th>
<th>250 ppm</th>
<th>500 ppm</th>
<th>750 ppm</th>
<th>0 ppm</th>
<th>250 ppm</th>
<th>500 ppm</th>
<th>750 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>470.00</td>
<td>470.00</td>
<td>470.00</td>
<td>470.00</td>
<td>470.00</td>
<td>470.00</td>
<td>470.00</td>
<td>470.00</td>
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<tr>
<td>Soyabean meal</td>
<td>260.00</td>
<td>260.00</td>
<td>260.00</td>
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<td>260.00</td>
<td>260.00</td>
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<td>Fish meal (72%)</td>
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</tr>
<tr>
<td>Wheat offal</td>
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<td>150.00</td>
<td>150.00</td>
<td>150.00</td>
<td>150.00</td>
<td>150.00</td>
<td>150.00</td>
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</tr>
<tr>
<td>Bone meal</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
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<tr>
<td>Oyster shell</td>
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</tr>
<tr>
<td>Salt</td>
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<tr>
<td>Methionine</td>
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<td>2.50</td>
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<tr>
<td>Lysine</td>
<td>2.50</td>
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<td>2.50</td>
<td>2.50</td>
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<td>2.50</td>
</tr>
<tr>
<td>Methionine (%)</td>
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<td>3.51</td>
<td>3.51</td>
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<td>3.51</td>
<td>3.51</td>
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</tr>
<tr>
<td>Ca (%)</td>
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<td>1.47</td>
<td>1.47</td>
<td>1.47</td>
<td>1.47</td>
<td>1.47</td>
<td>1.47</td>
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<tr>
<td>AV phos (%)</td>
<td>0.45</td>
<td>0.45</td>
<td>0.45</td>
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<td>0.45</td>
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<tr>
<td>Lysine (%)</td>
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<td>1.25</td>
<td>1.25</td>
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<td>1.25</td>
<td>1.25</td>
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<tr>
<td>Methionine (%)</td>
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<td>0.6</td>
<td>0.6</td>
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<td>0.6</td>
<td>0.6</td>
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</tbody>
</table>

Calculated Analysis

ME (MJ/kg⁻¹) 12.80 12.80 12.80 12.80 12.80 12.80 12.80 12.80
Crude Fibre 3.88 3.88 3.88 3.88 3.88 3.88 3.88 3.88
Crude Protein 20.00 20.00 20.00 20.00 20.00 20.00 20.00 20.00
Fat 3.51 3.51 3.51 3.51 3.51 3.51 3.51 3.51
Ca (%) 1.47 1.47 1.47 1.47 1.47 1.47 1.47 1.47
AV phos (%) 0.45 0.45 0.45 0.45 0.45 0.45 0.45 0.45
Lysine (%) 1.25 1.25 1.25 1.25 1.25 1.25 1.25 1.25
Methionine (%) 0.6 0.6 0.6 0.6 0.6 0.6 0.6 0.6

Table 3. Interaction effect of prebiotic sources and prebiotic levels of inclusion on the performance characteristics of finishing broilers.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lactose</th>
<th>Mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average initial weight (g)</td>
<td>666.67</td>
<td>660.0</td>
</tr>
<tr>
<td>Average final weight (g)</td>
<td>1370.0⁰</td>
<td>1356.67⁰</td>
</tr>
<tr>
<td>Total weight gain (g)</td>
<td>703.33⁰</td>
<td>716.67⁰</td>
</tr>
<tr>
<td>Total feed intake (g)</td>
<td>1356.67⁰</td>
<td>1356.67⁰</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>1.93⁰</td>
<td>1.89⁰</td>
</tr>
</tbody>
</table>

P<0.05 value of 50.00 um in birds fed 500 ppm levels of lactose inclusion. The trend observed in width (apical) of villus showed that birds fed 0 ppm lactose, 250 ppm mannose and 500 ppm mannose recorded higher values, and those on 500 ppm lactose had least (P<0.05) values. The report of intestinal morphology of the broiler finisher in the present study supported the works of Savage et al. (1996) and Iji et al. (2001) that MOS influenced the
physical properties of the epithelial lining by increasing the number of globlet cells with an inclusion level of mannose.

Conclusion

Higher final live weight, weight gain, improved FCR and villi height recorded for broilers fed 500 ppm mannann oligosaccharides (MOS) suggested the superiority of MOS over lactose (LOS) in supporting growth and gut morphology of broiler chickens.

REFERENCES


Other Journals

- Journal of Agricultural Science and Practice
- Research Journal of Food Science and Nutrition
- Journal of Practical Medicine and Medical Science
- Journal of Public Health and Diseases
- Global Journal of Earth and Environmental Science
- Journal of Drugs and Pharmaceutical Science
- Journal of Bioscience and Biotechnology Discovery
- Journal of Engineering Innovations and Applications
- Applied Journal of Physical Science
- Integrity Journal of Education and Training
- Research Journal of Business and Economic Management
Journal of Animal Science and Veterinary Medicine

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