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ORIGINAL ARTICAL **PAGE**

SHORT COMMUNICATION

BOVINE TRICHOMONIASIS: AN OVERVIEW

Adeyeye A A*[1], Ate I U[2], Bale J O[3], Lawal A I[4]
[1]Department of Veterinary Medicine, Surgery & Theriogenology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto-Nigeria.
[2]Department of Veterinary Surgery & Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria-Nigeria.
[3]National Animal Production Research Institute, Shika-Zaria, Nigeria.
[4]Department of Veterinary Parasitology & Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria-Nigeria.

Abstract

Bovine trichomoniasis is a sexually transmitted and host specific disease in cattle. It is caused Tritrichomonas foetus, a flagellated protozoa found in the reproductive tract particularly the prepuce and the distal penis in the bull and the vagina and uterus of the cow. It is worldwide in distribution although prevalence rates had subsided particularly in areas where artificial insemination is widely used. Bulls are asymptomatic chronic carriers of the organism and since infection does not affect the fertility of the bull or the viability of their spermatozoa, they are regarded as permanent sources of infection. In the cows, it is characterized by prolonged breeding interval; repeat breeding, early embryonic death, occasional late abortions and rarely pyometra. The asymptomatic nature of the disease makes accurate diagnosis difficult thereby posing serious economic losses. Information about the disease in Africa are few, the distribution of the disease particularly in Africa are discussed in the paper.

Keyword: Africa, Bovine, Infertility, Livestock, Production, Trichomoniasis.

TRICHOMONOSE BOVINE: UN APERÇU

Résumé

La trichomonose bovine est une maladie sexuellement transmissible des bovins, spécifique à l'hôte. Elle est causée par Tritrichomonas foetus, un protozoaire flagellé vivant dans l'appareil reproducteur, en particulier le prépuce et la partie distale du pénis du taureau et le vagin et l'utérus de la vache. On la retrouve partout dans le monde, bien que les taux de prévalence aient baissé, en particulier dans les zones où l'insémination artificielle est largement pratiquée. Les taureaux sont des porteurs asymptomatiques chroniques de l'agent étiologique, et puisque l'infection n'affecte pas leur fertilité ou la viabilité de leurs spermatozoïdes, ils sont considérés comme des sources permanentes de l'infection. Chez les vaches, elle est caractérisée par un intervalle de reproduction prolongé ; l'infécondité, une mortalité embryonnaire précoce, des avortements tardifs occasionnels et rarement le pyomètre. Le caractère asymptomatique de la maladie rend difficile un diagnostic précis, causant ainsi de graves pertes économiques. En Afrique, les informations sur la maladie sont rares, et sa répartition, en particulier sur le continent, est abordée dans le document.

Mots-clés: Afrique, Bovine, Infertilité, Elevage, Production, Trichomonose

*Corresponding author - ayo4wale@hotmail.com

Introduction

Trichomoniasis is a disease caused by various species of the Family Trichomonadidae in mammals and birds (Soulsby, 1982). Bovine trichomoniasis is an economic (Rae, 1989) host-specific and sexually transmitted disease (BonDurant *et. al.,* 1993). It is caused by Tritrichomonas foetus (Rhyan *et al.,* 1999), a flagellated protozoan that inhabits the reproductive tract particularly the prepuce and the distal penis in the bull and the vagina and uterus of the cow (Jubb *et al.,* 1985). The organism has been reported to cause diarrhoea in cats (Gookin *et al.,* 1999). In addition, the protozoan occur in pigs, horses and deers but pathogenic effects have not been reported (Soulsby, 1982).

Bovine trichomoniasis was first reported in France by Kunsler in 1888 and in Italy by Mazzanti in 1900 (Skirrow and BonDurant, 1988; Rae and Crews, 2006). However the discovery of brucellosis limited research into bovine trichomoniasis as a cause of infertility in cattle. Later, between 1924 and 1929, Drescher, Riedmiller and Abelein reported the disease in Germany (Rae and Crews, 2006). In 1932, the disease was reported in the United States of America (Emmerson, 1932) while Dumaresq reported it in Australia in 1948 (Skirrow and BonDurant, 1988). By 1946, 26 countries including South Africa had reported bovine trichomoniasis. In Nigeria, the occurrence of the disease was first reported by Akinboade (1980) from an abattoir survey. Ayoade *et al.,* (1990) later reported the disease at the University of Ibadan teaching and research farm. The prevalence of the disease has subsided in most countries where artificial insemination is practiced (BonDurant, 1985).

Cattle are found throughout Africa providing protein in meat, milk and blood as well as transportation in agriculture for ploughing, harrowing, ridging and lifting of water from deep wells (Blench, 1999). Diseases are regarded as a major hindrance to livestock production (Akerejola *et al.,* 1979; Lamorde, 1996). Bovine trichomoniasis is one of such diseases limiting livestock production. The gold standard for the diagnosis is culture media which is time consuming. Considering this and complex nature of diagnosis, an overview of the disease stressing recent diagnostic methods is important.

Aetiology

Bovine trichomoniasis is caused by Tritrichomonas foetus, a flagellated protozoan parasite of the bovine reproductive tract (BonDurant, 1985). It was formerly referred to as Trichomonas foetus (Riedmuller, 1978). The organism (Figure 1) tends to be pyriform measuring approximately 20 µm by 10 µm (Rae and Crews, 2006). T. foetus is characterized by one posterior and three anterior flagella with an undulating membrane running along the side of the organism (Skirrow and BonDurant, 1988) giving it a characteristic rolling, jerky motility that is diagnostic (BonDurant, 1985) when compared with other Trichomonad species found in the preputial cavity contaminated with faecal material during sampling (Rae and Crews, 2006).

Three serotypes have been identified based on agglutination reactions (Skirrow and BonDurant, 1988) namely belfast strain reported in Europe, Africa and the USA (Gregory *et al.,* 1990), the brisbane strain reported in Australia (Elder, 1964) and the manley strain, which has only been reported in few outbreaks (Skirrow and BonDurant, 1988). Antigenic and pathogenic differences do not exist between serotypes (Skirrow and BonDurant, 1988; Rae and Crews, 2006). Apart from this, there has been a report of cross-reaction among serotypes (Wosu, 1977).

Classification

The taxonomic position of Tritrichomonas foetus is based on the classification scheme outlined by Dyer (1990) in which the protozoon is classified as follows:

Phylum: Zoomastigina – possess flagella.
Class: Parabasalia – presence of a parabasal body associated with kinetosomes, undulating membrane, an extension of the plasma membrane enveloping the recurrent flagellum.
Order: Trichomonadida – four to six flagella, free or attached to an undulating membrane, no true cyst.
Family: Trichimonadidae – presence of a

cytostome, three to five flagella.
Genus: Tritrichomonas – presence of three anterior flagella.
Specie: Tritrichomonas foetus.

Apart from T. foetus, other pathogenic Trichomonads related to T. foetus are T. gallinae (in birds) and T. vaginalis (in humans). T. suis (in pigs) is the most common of other non-pathogenic Trichomonads probably due to its serological similarity with T. foetus (Skirrow and BonDurant, 1988). However, T. vaginalis is the most closely related Trichomonad with T. foetus (Schwebke and Burgess, 2004). It is a sexually transmitted disease (STD) in humans characterized by vaginitis and lower abdominal pain in females (Jatau *et al.,* 2006). Taylor *et al.* (1994) differentiated T. foetus from other medically non-important gut flagellates by either the absence or not well developed undulating membrane.

Epidemiology

Susceptibility

Breed, sex and age susceptibility have been reported as likely risk factors to the occurrence of Tritrichomonas foetus (Rae and Crews, 2006). BonDurant *et al.,* (1990) reported a higher prevalence among Bos taurus than Bos indicus. Within the B. taurus group, Rae *et al.,*(2004) reported that Simmental, Charolais and Angus were more likely to habour the organism but Herefords is assumed to be more resistant (Skirrow and BonDurant, 1988). In Nigeria, the disease has been reported in the Bunaji, Sokoto Gudali, Keteku, Red Bororo (Akinboade, 1980), N`Dama and their crosses (Ayoade *et al.,* 1990).

There is no major difference in the susceptibility of the male and female cattle. However, once a male is infected it remains a carrier for life (BonDurant, 1985). Most females undergo "self cure", although the organism persist in some cows and they are able to carry their pregnancy to term without abortion (Skirrow, 1987). The prevalence rate in bulls is reported to be higher than in the cow (Akinboade, 1980; Rae and Crews, 2006). Although the duration of infection in the cow and bull is not fully known, experimental infections in cows have lasted for between 8 weeks to 19.5 weeks (Skirrow and BonDurant, 1990; BonDurant *et al.,* 1993) and the duration

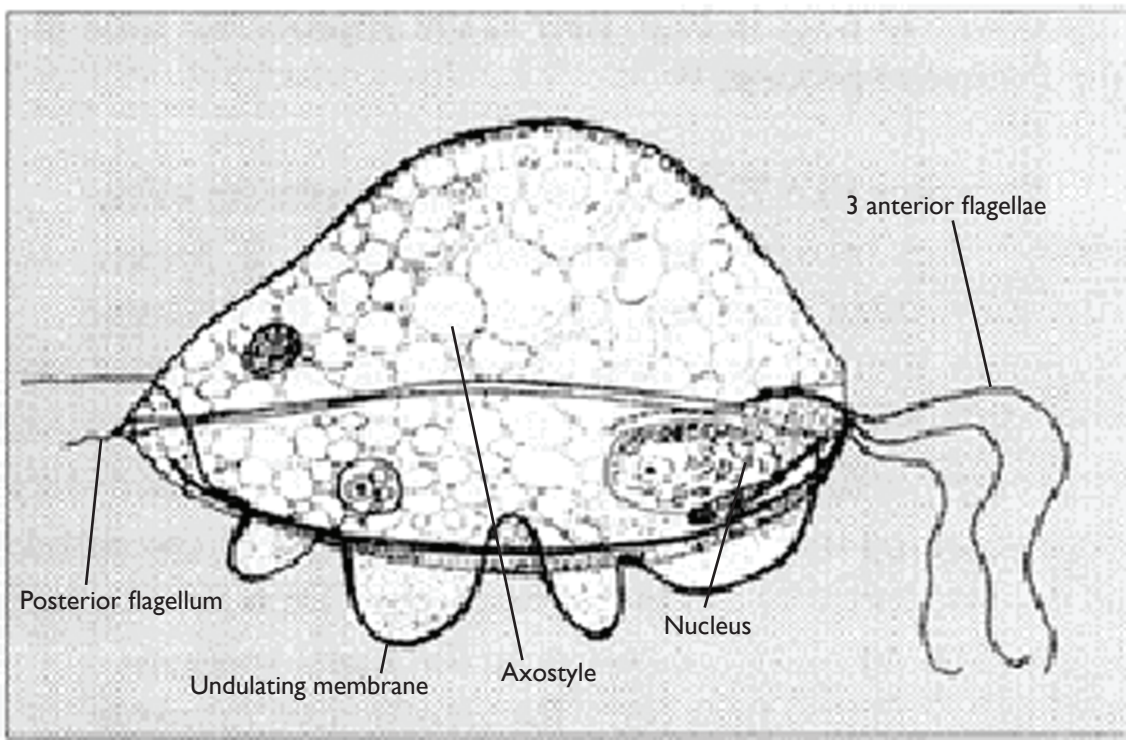

Figure 1: A photomicrograph of Tritrichomonas foetus (Source - Thomas and Harmon, 1994). Classification

of infection in the bull is reported to be much longer than in the cow (Corbeil, 1999). The ability of the organism T. foetus to persist in the crypts of the external mucous membrane of the penis and prepuce (Anon, 2010) may account for the carrier state in the male (BonDurant, 1985). Apart from this, the difference in local immune response of the male and female genital mucosa (BonDurant *et al.,* 1993) may be another contributory factor.

Bovine trichomoniasis increases with age in the bull (Skirrow and BonDurant, 1988). Field surveys suggest that older bulls become more permanent carriers than younger bulls due to the development of epithelial crypts (BonDurant, 1985). Bulls 2-4 years old carry the infection for a short period while bulls 4 years and above are asymptomatic carriers for life (Corbeil, 1999). Younger bulls 1-2 years are not resistant to the disease as infection has been reported within the age group (Ayoade *et al.,* 1990). Sexually active females of all ages are equally susceptible (Irons *et al.,* 2004) but age disposition has not been reported in females.

Other factors associated with increased prevalence of the disease are sexual rest, communal grazing and exposure to greater number of other herds (Irons *et al.,* 2004). BonDurant, (1985) had recommended a sexual rest period of 1-2 weeks for bulls before sampling to enable a build up of the organism in the preputial and penile epithelium. Communal grazing and exposure of animals to herds provides opportunities for the organism to spread following uncontrolled natural mating within the herds (Perez *et al.,* 1992).

Transmission

Transmission is by natural breeding of an infected bull with a susceptible cow/ heifer or an infected cow with a susceptible bull (BonDurant, 1985). Passive transmission (transmission by an uninfected bull to an uninfected cow after mating an infected female) has been reported (Rae and Crews, 2006). Rarely, mechanical transmission by equipment used for artificial insemination, examination of the genital tracts as well as contaminated semen can transmit the disease (Goodger and Skirrow, 1986). Non-venereal transmission by flies has been speculated but no evidence to substantiate it (Skirrow and BonDurant, 1988).

Distribution

Bovine trichomoniasis is worldwide in distribution (Gookin *et al.,* 1999; Lun and Gajadhar, 1999). It is associated with serious economic losses and high prevalence exist particularly in areas where natural breeding is practised (Rae, 1989; Corbeil, 1999). The disease has been reported in Europe, America, Australia, Asia (Skirrow and BonDurant, 1988) and Africa (Akinboade, 1980; Irons *et al.,* 2004). Prevalence rates have drastically subsided due to wide use of artificial insemination in Britain where the disease is assumed to be eradicated (Urquhart *et al.,* 2003). However, in developing countries of Africa where artificial insemination is not widely practised, the prevalence may be considerably high (OIE, 2008). In these countries, the disease is not often reported due to poor reporting system and the complex nature of making diagnosis which is time consuming coupled with the insidious nature of Tritrichomonas foetus in a herd (Irons, 2002; Anon., 2010). Klastrup and Halliwell (1977) failed to demonstrate the disease from 348 samples collected at a breeding centre in Malawi and concluded that the disease was not present in any significant level. Swai *et al.,* (2005) gave a similar report that bovine trichomoniasis is not an endemic cause of infertility in Tanzania. However, Gawade et al. (1981) found an incidence of 4.6 % among Holstein bulls in Egypt while prevalence rates of 7.1% (Pefanis *et al.,* 1988) to 26.4% (Erasmus *et al.,* 1989) had earlier been reported in South Africa, Kitching (1999) and Ribiero (1999) later reported a prevalence of 1.8 % and 25 % respectively. However, Madoroba *et al.,* (2011) reported a prevalence of 4.1 %.

From available literature, Akinboade, (1980) first reported the occurrence of the disease in Nigeria from an abattoir survey using wet preparation (direct microscopy) method, he found a prevalence of 71% from 200 cattle in a settled herd and 14.9% from 960 trade cattle slaughtered in Ibadan. Ayoade *et al.,* (1990) later reported a prevalence of 100 % from bulls in a herd with history of infertility. However, Adeyeye *et al.,* (2010), reported a zero prevalence in cattle slaughtered at the

Sokoto metropolitan abattoir, Sokoto-Nigeria using both direct microscopy and culture. They therefore concluded that the disease maynot be present in the animals slaughtered within the study period.

Pathogenesis

In the male, Tritrichomonas foetus is found only in the mucosa surface of the preputial cavity (Skirrow and BonDurant, 1988), which is the primary site for infection in the bull (Soulsby, 1982; Skirrow and BonDurant, 1988). However, it has also been isolated from other parts of the male genital tract (Irons *et al.,* 2004) such as the urethral orifice (Rhyan *et al.,* 1999). Higher number of the organism are found on the penile mucosa and adjacent posterior preputial mucosa (Hammond and Bartlett, 1943) where they localize in the secretions of the epithelial lining of the penis, prepuce and distal urethra (Rae and Crews, 2006). In early stage of infection, balanoposthitis may occur, leading to painful urination and refusal to serve cows (Soulsby, 1982; Jubb *et al.,* 1985). A mucoid to mucopurulent discharge may be present on the mucous membrane of the prepuce and the glans penis (Soulsby, 1982); with time these signs disappear but bulls above 4 years of age become persistently infected (Skirrow and BonDurant, 1988; Rhyan *et al.,* 1999).

Pathogenesis of trichomoniasis in the female has not been fully determined (Skirrow and BonDurant, 1988). However, it is postulated that exposure is through natural service by a carrier bull or artificial insemination with contaminated semen (Anon. 2010). T. foetus is found on the endometrial surface and in endometrial glandular lumina (Andreson *et al.,* 1996). The organism first multiplies in the vagina and cervix for about 3 weeks (Anon. 2010) causing vaginitis (Soulsby, 1982). Later, it invades the uterus through the cervix during estrus from where it gets to the oviduct (BonDurant, 1985). Within 1-2 weeks, the organism colonizes the whole reproductive tract (Rae and Crews, 2006). After invading the uterus, a few "carrier cows" may conceive and carry their pregnancy to term, giving birth to healthy calf (Skirrow, 1987). In others, it interferes with fertilization and development of the embryo (Clark *et al.,* 1983) causing early embryonic death and placentitis (Anon. 2010) but rarely late abortions (Soulsby, 1982). Occasionally, after foetal loss, if corpus luteum (CL) of pregnancy is maintained, pyometra develops (Rae and Crews, 2006) containing large volumes of thin, greyish-white odourless material rich in T. foetus (Irons et al., 2004). The organism has also been found in foetal oesophagus, abomasums and intestine (Rae and Crews, 2006).

Pathology

There is usually no gross evidence of trichomoniasis in the bull (Skirrow and BonDurant, 1988) except for a mild swelling of the prepuce and slight preputial discharge seen within the first 2 weeks of the infection (Clark *et al.,* 1983). Debris accumulates at the crypts of the mucosa although T. foetus cannot be found there (Irons et al., 2004). Histologically, mild cellular infiltration characterized by inflammatory reaction is seen in the sub-epithelium of the penis and prepuce (Skirrow and BonDurant, 1988). Flower *et al.* (1982) observed frequent plasma cells in the preputial dermis of infected bulls.

Few lesions are seen in the female within the first 50 days of infection (Irons *et al.,* 2004). However, varying degrees of vaginitis, cervicitis, endometritis and salpingitis are seen in most cases between 50 to 100 days of infection (Anderson *et al.,* 1996). Aborted foetuses have oedematous placentas but the foetuses may be fresh or autolysed (Rhyan *et al.,* 1995). Foetuses aborted in late gestation show pyogranulomatous, necrotizing enteritis and bronchopneumonia with identifiable trichomonads in their airways (Irons *et al.,* 2004; Rae and Crews, 2006). Histologically, placenta lesions have stromal oedema, mixed inflammatory cell infiltration and focal necrosis of the chorionic epithelium (Jubb *et al.,* 1985; Rae and Crews, 2006).

Clinical Signs

Trichomoniasis is asymptomatic in the bull (BonDurant, 1985; Grotelueschen *et al.,* 1994; Rhyan *et al.,* 1999) but cows and heifers shed the organism after 5-20 weeks (Skirrow and BonDurant, 1990). However, following infection in the cow there is mild vaginitis,

placentitis, salpingitis, cervicitis, endometritis leading to mucopurulent vaginal discharge (Skirrow and BonDurant, 1988). Foetal loss and inflammation occur between 60-90 days (Corbeil, 1999) but from field cases, foetal loss has been reported to occur from days 16-17 through 7 months of gestation (BonDurant, 1985). A cow may remain infertile for up to 6 months before fertility is regained (Corbeil, 1999). There is extended breeding periods resulting in increased inter-calving intervals and reduced calving rates (Clark *et al.,* 1986). Palpation of post-coital pyometra during pregnancy examination is highly suggestive of trichomoniasis (Skirrow and BonDurant, 1988). Pyometra and abortion are the first physical signs of infection in a herd (Rae and Crews, 2006). Unlike abortion, pyometra is seen in a few cows following fetal loss (Skirrow and BonDurant, 1988) although it is rare. The disease is more insidious in dairy herds (Ball *et al.,* 1984) with an increase in "repeat breeder", services per conception and abortions (Goodger and Skirrow, 1986). History of repeat breeding can also enhance diagnosis of the disease in a herd (Ayoade *et al.,* 1990).

Immunology

The bull does not develop an immune response to T. foetus since the organism continues to live in the penis and prepuce (Kimberling *et al.,* 2009). However, in the female, three antibodies are produced by Tritrichomonas foetus (Soulsby, 1982).

a. Circulating antibody – It is stimulated by large numbers of the organism in the uterus following field infections associated with cases of abortion and pyometra (Soulsby, 1982). It has also been induced by experimental infections and systemic immunization of cows with the parasite although the immunity is short lived (Skirrow and BonDurant, 1988). After initial infection, the cow will develop an immune response and free itself of the parasite (BonDurant *et al.,* 1996). The next pregnancy will go undisturbed (Kimberling *et al.,* 2009). Recent studies on the immunology of bovine trichomoniasis suggest that the antibody response in serum is predominantly IgG1 and IgG2 (BonDurant *et al.,* 1993). Serum antibodies derived post-infection have low protective value compared to locally synthesized antibodies which are responsible for the elimination of the parasite from the reproductive tract (Soto and Parma, 1989).
b. Uterine antibody developing in situ – This antibody is produced in the uterus and is responsible for clearing the organism from the uterus in mild infection (Soulsby, 1982). In such infections, pregnancy is usually not interrupted but carried to term.
c. Vaginal antibody - It develops locally independent of the circulating and uterine antibodies (Soulsby, 1982). The antibodies are able to agglutinate and probably eliminate the parasite from the vagina but cannot immunize it from re-infection (Skirrow and BonDurant, 1988). Experimental infection of the vagina with T. foetus has been reported to induce infections of variable duration (up to 32 weeks), and clearance of the parasite from the genital tract is associated with the appearance of parasite-specific immunoglobulin G1 (IgG1) and IgA antibodies in vaginal mucous and uterine secretions (BonDurant *et al.,* 1993; Ikeda *et al.,* 1995).

Diagnosis

Diagnosis of bovine trichomoniasis is difficult due to the insidious nature of the disease (Rae and Crews, 2006). Tentative diagnosis in a herd is based on clinical signs of early abortions, repeat breeding, prolonged breeding interval and occasionally late abortions and pyometra (BonDurant, 1985; OIE, 2008). Bulls are the best animals to be sampled except if aborted foetuses or females with pyometra are available (Irons *et al.,* 2004). The disease is differentiated from other causes of reproductive losses such as campylobacteriosis by the demonstration of the organism in the preputial cavity of the bull (Irons, 2002).

Sampling methods

There are three methods of collecting samples from the preputial cavity of the bull and vagina of the cow (Rae and Crews, 2006). These are: swabbing, washing and scrapping. Whichever method used, it is important to

vigorously scrape the surface of the penis and prepuce to dislodge the organism from the epithelial crypts (Skirrow and BonDurant, 1988).

Swabbing: In the bull, the prepuce is first cleaned after which a sterile swab stick or gauze-covered rod moistened with physiological buffered saline (PBS) is used to swab the crypts of the penis and preputial mucous membrane (Akinboade, 1980; Skirrow and BonDurant, 1988). Similarly, in the cow the vulval labiae are parted using vaginal speculum and a sterile swab stick moistened with PBS (Ball *et al.,* 1984) is inserted into the vulva or vagina without contamination from the external genitalia to swab the vagina and the caudal part of the cervix.

Washing (douching): It involves infusing 50 mls of sterile PBS into the preputial cavity of the bull using a funnel and flexible tube (Irons *et al.,* 2004). Vigorous massaging of the prepuce will retrieve an opaque sample that contains cellular debris (Schonmann et al., 1994). In the female, samples are collected by washing the vagina with PBS (OIE, 2008). Sheath washing is practised more in Europe due to the advantage of recovering materials from the whole preputial cavity (Irons, 2002).

Scrapping: A sterile artificial insemination (AI) pipette connected by means of a short silicon tubing to a hypodermic syringe is inserted into the prepuce to the level of the fornix in the male (Irons *et al.,* 2004), and into the vagina to the level of the cervix in the female (Rae and Crews, 2006). The pipette is vigorously scrapped back and forth across the preputial epithelia and cervix for about 30 to 45 times after which suction is applied to withdraw smegma in the male (Irons *et al.,* 2004) and cervico-vagina mucous in the female (Rae and Crews, 2006). The scrapping can be very vigorous to the extent that blood may be seen suggesting the depth of the scrapping (Irons *et al.,* 2004). Sheath scrapping is the preferred method in the United States (Rae and Crews, 2006).

Samples collected by swabbing and washing are centrifuged to concentrate the sample before examination by wet mount or inoculation into culture media. The methods of sampling have been researched into by several investigators across the globe (Irons 2002). However, OIE (2008) recommends either sheath washing or scrapping since the two methods do not differ significantly. However, Irons et al. (2002) concluded from an experimental research that sheath scrapping is faster, safer and more advantageous over sheath washing.

Once collected, samples should be processed immediately (BonDurant, 1985). If this is not possible, it may be chilled if collected in plastic pipette and transported to the laboratory (Irons *et al.,* 2004) or placed in a transport medium containing antibiotics where delivery must be within 24 hrs (OIE, 2008). The sample can be inoculated immediately after collection into a transport culture medium of refrigerated buffered saline containing lactated Ringer`s solution, Kupferberg medium and milk-based media. The medium is enriched with bovine foetal serum although it maintains viability for 48 – 96 hrs (Skirrow *et al.,* 1985; Skirrow and BonDurant, 1988). It is important during transport that the samples are protected from daylight as well as ensuring temperatures do not exceed 38^0C nor fall below 5^{0C} (OIE, 2008). Despite the transport medium, some sensitivity is lost when incubated after 24 hours (Irons, 2002).

Diagnosis techniques

Direct examination: Following sample collecting as earlier described, either the sediment from the centrifuged swabs or washings, or the sample from the scrappings is placed on a grease-free glass microscopic slide and observed with or without coverslip at x100 or x400 magnification (Irons et al., 2004). The organism is recognized by its jerky motility (BonDurant, 1985). Sensitivity of the direct microscopic test is generally low as non-detection of the disease in animals with low number of the organism may give a false negative (Ribiero, 1999).

Culture media and methods: Culturing is the gold standard for the diagnosis of bovine trichomoniasis (BonDurant *et al.,* 2003). Culture media available are diamond trichomonad medium, commercial culture kits, Cysteine/peptone/liver-infusion maltose medium (CPLM), Beef-extract/glucose/peptone

serum medium (BGSPS), Clausen`s medium (Neopeptone-Lemco-liver extract glucose) and Oxoid trichomonas medium (OIE, 2008). It involves placing sediments from centrifuged swabs or washings, or samples (smegma or cervico-vagina) from the scrappings and inoculating into culture media (BonDurant, 1985) after which the media is incubated at 22^{0}C and 37^{0}C (Rae and Crews, 2006). Diagnosis can be made from the bottom of the media by wet mount within 48 hours of inoculation (Irons *et al.,* 2004) but wet mount should be made for 7 days (Lun *et al.,* 2000). This is done by placing the media directly on a grease-free microscopic slide and observing with or without cover slip at x10 or x40 magnification (BonDurant, 1985). The principle of the culture is to provide an environment for the organism to multiply (OIE, 2008). This diagnostic test is better than direct examination as it is about 98% sensitive (Schonmann *et al.,* 1994). However; time, temperature, type of isolate and type of media affect the sensitivity of T. foetus in culture (Rae and Crews, 2006). Cultures are usually overgrown with contaminants despite the presence of anti-microbial agents (Irons *et al.,* 2004). Bulls are tested three consecutive times at an interval of 1-2 weeks before they are declared free of trichomoniasis (BonDurant, 1997; Parker *et al.,* 1999).

A field culture test kit called in-pouch, which is conveniently used to collect samples in the field (OIE, 2008) without requiring a transport medium has been developed (Irons *et al.,* 2004). Samples for in-pouch are usually collected by scrapping (Schonmann *et al.,* 1994) since centrifugation cannot be done outside the laboratory. The kit has a clear flexible plastic pouch with an upper chamber containing special medium where samples are introduced and forced into the lower chamber after mixing and sealed for incubation 37^{0}C for 7 days like the culture media (OIE, 2008). Microscopic examination is through the plastic pouch at x100 or x400 magnification (Borchardt *et al.,* 1992). The kit has a sensitivity of about 92% (Parker *et al.,* 1999) with an advantage of facilitating transport, incubation and examination in situation where sophisticated facilities are not available (Irons *et al.,* 2004). It is also faster to use than the culture media (Borchardt *et al.,* 1992).

Serology: Attempts have been made to diagnose trichomoniasis in cattle by serology (Irons *et al.,* 2004) but little systemic immune response is stimulated by the organism following infection particularly in the bull (Skirrow and BonDurant, 1990; BonDurant *et al.,* 1993). This may be attributed to their role as carriers.

Vaginal mucus agglutination involving mucus from the cervical region of the vagina, collected few days after estrus were used to diagnose the disease in cows (Kerr and Robertson, 1941; Pierce, 1949). This test lacks sufficient specificity for accurate diagnosis (Reece et al., 1981) although it is useful in diagnosing the presence of existing infection as well as cleared infections (OIE 2008). ELISA has been demonstrated to detect long lasting IgA in vaginal mucous from 6 weeks post-infection in cows and heifers (Ikeda *et al.,* 1995) with sensitivity and specificity of 85% and 95% respectively (BonDurant, 1997), But the test cannot diagnose the disease in bulls (Irons *et al.,* 2004).

Kerr (1944) reported the diagnosis of bovine trichomoniasis by intradermal test similar to tuberculin test although Soto and Parma (1989) concluded that this can only be used as a screening test for females. Tritrichomonas foetus has been recovered from aborted foetus with an immunohistochemical technique using a monoclonal antibody (Rhyan *et al.,* 1995). In that study, the organism was detected in formalin-fixed, paraffin-embedded sections of placenta and foetal lungs from bovine abortion.

Polymerase Chain Reaction (PCR): It involves the extraction and amplification of DNA using PCR techniques with specific primers (OIE. 2008). Primers specific for sequencing in the 5.8S ribosomal RNA gene have been demonstrated to be better in its sensitivity and specificity (Felleisen, 1997). The test can be used to detect T. foetus DNA in formalin-fixed endometrial and aborted tissues (BonDurant *et al.,* 2003). Apart from this, it has faster diagnostic time, high specificity, ability to analyse large numbers of samples simultaneously and the fact that the organisms are not required to be viable to detect T. foetus (Morgan and Thompson, 1998). However, the presence of contaminants in

the vagina and preputial cavity during sample collection (Irons, 2002) as well as components of blood from sheath scrapping and urine during sheath washing reduce the sensitivity of PCR (Irons, 2002). Campero *et al.,* (2003) recommends a two-step (culture and PCR) diagnostic approach in bovine trichomoniasis. It involves subjecting positive samples from culture to PCR.

Treatment

There is no effective drug for treating the disease (BonDurant, 1985; Kimberling, 2009). Acriflavine, diminazene aceturate, chlorhexidine, imidazole and metronidazole were used in the past (Skirrow and BonDurant, 1988) but since the disease is self-limiting in the cow (Soulsby, 1982), treatment may not be necessary. Initially, ipronidazole followed by 2-4 days broad-spectrum antibiotic has been reported to be effective in South Africa (BonDurant, 1985). However, none of these anti-protozoan drugs is approved for use in the United States of America (BonDurant, 1985; Skirrow and BonDurant, 1988).

Prevention and control

Prevention and control of trichomoniasis is based on herd health programme (Rae and Crews, 2006). However, the following measures described by several investigators as possible ways of preventing and controlling the disease are enumerated below:

a. Control of movement of animals in and out of the herd (BonDurant, 1985) will prevent the introduction of the disease to a herd.
b. Stoppage of communal grazing of cattle (Rae and Crews, 2006).
c. Use of only virgin bulls or heifers for replacement (Parsonson *et al.,* 1974).
d. Culture of samples from new entry bulls including virgin bulls (Rae and Crews, 2006).
e. Separation of newly purchased cows and heifers during breeding to reduce risk of exposure from infected cows (Rae and Crews, 2006).
f. Vaccination using either the commercially available monovalent or polyvalent vaccine containing Campylobacter and Leptospira spp (OIE, 2008). They are effective only in the female but not males (BonDurant *et al.,* 1993). Currrently, TrichoGuard (Fort Dodge) is available in the United States of America.
g. Use of artificial insemination in lieu of natural breeding (BonDurant, 1985; Irons *et al.,* 2004).
h. Use of young bulls for breeding in a herd since age has been incriminated as a predisposing factor (Skirrow and BonDurant, 1988; Rae *et al.,* 1999).
i. Cows within a herd that abort should be given sexual rest (Rae and Crews, 2006)

Conclusions and Recommendations

Bovine trichomoniasis is a sexually transmitted host specific disease of cattle that continue to pose a serious economic loss on cattle production due to infertility and abortion. The asymptomatic nature of the disease particularly in the bull makes diagnosis complex and difficult. Information about the disease is Africa is lacking and disjointed, herd survey to determine the status of the disease across the African continent is therefore recommended.

References

Adeyeye A A, Ate I U, Bale J O and Lawal A I. 2010. Survey for bovine trichomoniasis in cattle at slaughter in the Sokoto metropolitan abattoir Sokoto state, Nigeria. *Sokoto Journal of Veterinary Sciences,* 8(1&2): 18-21.

Anderson M L, BonDurant R H, Corbeil R R and Corbeil L B. 1996. Immune and inflammatory responses to reproductive tract infection with Tritrichomonas foetus in immunized and control heifers. *Journal of Parasitology*, 82: 594-600.

Anonymous. 2010. Infectious causes of infertility. In: Infertility in cows. Visited January 29, 2010 from http://www.ilri.org/InfoServ/Webpub/Fulldocs/X5442e/x5442e07.htm.

Akinboade O A. 1980. Incidence of bovine trichomoniasis in Nigeria. Revue d'élevage et de médecine vétérinaire des pays tropicaux, 33(4): 381-384.

Ayoade G O, Agbede S A and Ogungbade S G. 1990. Occurrence of Trichomonas foetus (Rieduller 1928) in settled cattle herd in Ibadan Nigeria. *Tropical Animal Production Investigation.* 1: 123-125.

Ball L, Mortimer R G, Cheney J M and Olson J D. 1984. Trichomoniasis: diagnosis, pathogenesis, treatment and control. Bovine Practitioner, 16: 163-165.

BonDurant R H. 1985. Diagnosis, treatment and control of bovine trichomoniasis. *The Compendium for Continuing Education for the Practicing Veterinarian*, 7: S179-S188.

BonDurant R H, Anderson M L, Blanchard P, Hird D, Danaye E C, Palmer C, Sischo W M, Suther D, Utterback W and Weigler B J. 1990. Prevalence of trichomoniasis among California beef herds. *Journal of American Veterinary Association*, 196: 1590-1593.

BonDurant R H, Corbeil R R and Corbeil L B. 1993. Immunization of virgin cows with surface antigen TF 1.17 of Tritrichomonas foetus. *Infection and Immunity,* 61(4): 1385-1394.

BonDurant R H, VanHoosear K A, Corbeil L B and Bernoco D. 1996. Serological response to in vitro-shed antigens(s) of Tritrichomonas foetus in cattle. *Clinical and Diagnostic Laboratory Immunology,* 3: 432-437.

BonDurant R H. 1997. Pathogenesis, diagnosis and management of trichomoniasis in Cattle. *Veterinary Clinics of North American food Animal Practice,* 13: 345-361.

BonDurant R H, Campero C M, Anderson M L and Van Hoosear K A. 2003. Detection of Tritrichomonas foetus by polymerase chain reaction in cultured isolates, cervicovaginal mucus, and formalin-fixed tissues from infected heifers and foetuses. *Journal of Veterinary Diagnosis Investigation,* 15: 579-584.

Borchardt K A, Norman B B, Thomas M W and Harmon W M. 1992. Evaluation of new culture method for diagnosing Tritrichomonas foetus. *Veterinary Medicine*, 87: 104-112.

Campero C M, Rodriquez Dubra C, Bolondi A, Cacciato C, Cobo E, Perez S, Odeon A, Cipolla A and BonDurant R H. 2003. Two-step (culture and PCR) diagnostic approach for differentiation of non-T. foetus trichomonads from genitalia of virgin beef bulls in Argentina. *Veterinary Parasitology*, 122: 167-175.

Clark B L, Dufty J H and Parsonson I M. 1983. The effect of Tritrichomonas foetus on calving rates in Beef cattle. *Australian Veterinary Journal,* 60(3): 71-74.

Clark B L, Dufty J H and Parsonson I M. 1986. The frequency of infertility and abortion in cow infected with Tritrichomonas foetus. Australian *Veterinary Journal,* 63(3): 31-32.

Corbeil L B. 1999. Immunization and diagnosis in bovine reproductive tract infections. *Advances in Veterinary Medicine*, 41: 217-239.

Dyer B, 1990. Phylum Zoomastigina Class Parabasalia. In Handbook of Protoctista, Eds., Margulis L, Corliss JO, Malkonian M and Chapman DJ: Jones and Barlett, Boston, Mass. pp: 252-258.

Elder J K. 1964. Examination of twelve strains of Tritrichomonas foetus (Reidmuller) isolated in Queensland and the description of a new serotype. T. foetus var. brisbane, *Queensland Journal of Agricultural Science,* 21: 193-203.

Emmerson M A. 1932. Trichomoniasis in cattle. *Journal of American Veterinary Medical Association,* 34: 636-640.

Erasmus J A, De Wet J A, Vander Merwe H E and Pienaar G C. 1989. Bovine trichomoniasis in the north western cape province,western Transvaal and the orange free state. *Journal of South African Veterinary Medical Association,* 60 (1): 51-52.

Felleisen B S J. 1997. Comparative sequences analysis of 5.8S rRNA genes and internal transcribed spacer (ITS) regions of trichomonadid protozoa. *Parasotology*, 115: 111-119.

Flower P J, Ladds P W, Thomas A D and Watson D L. 1982. An immunopathologic study of the bovine prepuce. *Veterinary Pathology,* 20: 189-202.

Gawade A F A, Nada S M and Micheal D G. 1981. Incidence of Trichomonas foetus infection in bulls in Sharkya Governorate, Egypt. *Assiut Veterinary Medicine Journal*, 8: 89-91.

Goodger W J and Skirrow S. 1986. Epidemiology and economic analysis of an unusually long epizootic of Trichomoniasis in a large California Dairy herd. *Journal of American Veterinary Medical Association,* 189: 772-776.

Gookin J L, Breitschwerdt E B, Levy M G, Gager R B and Benrud J G. 1999. Diarrhea associated with trichomonosis in cats. *Journal of American Veterinary Medical Association,* 215 (10): 1450-1454.

Gregory M W, Ellis B and Redwood D W. 1990. Comparison of sampling methods for the detection of Tritrichomonas foetus. *Veterinary Records*, 127: 16.

Grotelueschen D M, Cheney J, Hudson D B, Schweitzer D J, Kimberling C V, Taton-Allen G F, Nielsen K A and Marsh D J. 1994. Bovine trichomoniasis: Results of a slaughter survey in Colorado and Nebraska. Theriogenology, 42: 165-171.

Hammond D M and Bartlett D E. 1943. The distribution of Trichomonas foetus in the preputial cavity of infected bulls. *American Journal of Veterinary Research,* 4: 143-149.

Ikeda J S, BonDurant R H, Corbeil L B. 1995. Bovine vaginal antibody response to immunoaffinity-purified surface antigen of Tritrichomonas foetus. *Journal of Clinical Microbiology,* 33: 1158-1163.

Irons P C. 2002. Diagnosis of Tritrichomonas foetus in bulls by culture and PCR methods, Unpublished MMedVet Thesis, University of Pretoria, South Africa.

Irons P C, Henton M M and Bertschinger H J. 2002. Collection of preputial material by scrapping and aspiration for the diagnosis of Trichomonas foetus in Bulls. *Journal of South African Veterinary Association.* 73(2): 66-69.

Irons P C, Schutte A P, Herr S and Kitching J P. 2004. Trichomonosis. In: Coetzer J.A.W. and Tustin, R.C. (Eds), Infectious Diseases of Livestock. Oxford University Press. pp 305-315.

Jatau E D, Olonitola O S and Olayinka A T. 2006. Prevalence of Trichomonas infection among Women attending antenatal clinics in Zaria, Nigeria. *Annals of African Medicine*. 5 (4): 178-181.

Jubb K V F, Kennedy P C and Palmer N. 1985. Pathology of Domestic Animals. Academic Press, Orlando Florida.

Kerr W R and Robertson M. 1941. An investigation into the infection of cows with Trichomonas foetus by means of agglutination reaction. *Veterinary Journal,* 97: 351-363.

Kerr W R. 1944. The intradermal test in bovine trichomoniasis. Veterinary Record, 56: 303-305.

Klastrup N O and Halliwell R W. 1977. Infectious causes of infertility/abortion of cattle in Malawi. Nordisk *Veterinaer Medicin*, 29 (7-8): 325-330.

Kimberling C V, Cheney J and Cunningham W. 2009. Bovine trichomoniasis: The "silent rustler". Visited May 20, 2009 from http://www.colostate.edu/Depts/CoopExt/Adams/ag/pdf/bovine1.pdf.

Kitching J P. 1999. Trichomoniasis in Natal beef herds: importance, eradication and control. *Livestock Health and Production Group*, 2000: 9-10.

Lun Z and Gajadhar A A. 1999. A simple and rapid method for staining Tritrichomonas foetus and Trichomonas vaginalis. *Journal of Veterinary Diagnostic investigation,* 11: 471-474.

Lun Z, Parker S and Gajadhar A A. 2000. Comparison of the grow rates in Tritrichomonas foetus isolates and from various geographical regions using three different culture media. *Veterinary Parasitology,* 89: 199-208.

Madoroba E, Gelaw A, Hlokwe T and Mnisi M. 2011. Prevalence of Campylobacter foetus and Trichomonas foetus among cattle from Southern Africa. *African Journal of Biotechnology,* 10 (50): 10311-10314

Morgan U M and Thompson R C A. 1998. Molecular detection of parasitic protozoa. *Parasitology Supplement,* 117: S73-S85.

Office of the International Epizootic (OIE) 2008. Trichomoniasis. *In OIE Terrestrial Manual,* pp: 805-812.

Parker S, Campbell J R, Ribble C and Gajadhar A A. 1999. Comparison of two sampling tools for diagnosis of Tritrichomonas foetus in bulls and clinical interpretation of culture results. Journal of American Veterinary Medical Asssociation, 215: 231-235.

Parsonson I M, Clark B L and Dufty J H. 1974. The pathogenesis of Tritrichomonas foetus infection on bull. *Australian Veterinary Journal*, 50: 421-423.

Pefanis S M, Herr S, Venter C G, Kruger L P, Queirogan C C and Amaral L. 1988. Trichomoniasis and Campylobacteriosis in bulls in the Republic of Transkei. *Journal of South African Veterinary Medical Association,* 59(3): 139-140.

Perez E, Conrad P A, Hird D, Ortuno A, Chacon J, BonDurant R and Noordhuizen J, 1992. Prevalence and risk factors for Trichomonas foetus infection in cattle in north-eastern Costa Rica. *Preventive Veterinary Medicine,* 14: 155-165.

Pierce A C. 1949. The mucus agglutination test for the diagnosis of bovine trichomoniasis. *Veterinary Record,* 61: 347-349.

Rae D O. 1989. Impact of Trichomoniasis on the cow-calf producer`s profitability. *Journal of American Veterinary Medical Association,* 194: 771-775.

Rae D O, Chenoweth P J, Genho P C, McIntosh A D, Crosby E and Moore S A. 1999. Prevalence of Tritrichomonas foetus in a bull population and effect on production in a large cow-calf enterprise, *Journal of American Veterinary Medical Association,* 214: 1051-1055.

Rae D O, Crews J E, Griener E C and Donovan G A, 2004. Epidemiology of Tritrichomonas foetus in beef bull populations in Florida. *Theriogenology*, 61: 605-618.

Rae D O and Crews J E. 2006. Tritrichomonas foetus. *Veterinary Clinics, Food Animal Practice*. 22: 595-611.

Reece R L, Dennet D P and Johnson R H. 1981. Tritrichomonas foetus agglutination test upon samples collected from cattle: Cross-reactions associated `with vaccination against Campylobacter foetus subsp venerealis. *Australian Veterinary Journal*, 57: 352-353.

Ribiero L M M. 1999. An efficient medium for the isolation of Tritrichomonas foetus. *Onderstepoort Journal of Veterinary Research,* 57: 209-210.

Riedmuller L. 1978. Tritrichomonas foetus. In Parasitic Protozoa, Ed., Kreier, JP: New York: Academic Press, pp: 163-273.

Rhyan J C, Wilson K L, Burgess D E, Stackhouse L L

and Quinn W J. 1995. Immunohistochemical detection of Tritrichomonas foetus in formalin-fixed, paraffin-embedded sections of bovine placenta and foetal lungs. *Journal of Veterinary Diagnostic Investigation,* 1: 98-101.

Rhyan J C, Wilson K L, Wagner B. Anderson M L, BonDurant R H, Burgess D E, Mutwiri G K and Corbeil L B. 1999. Detection of Tritrichomonas foetus in the external genitalia and specific antibodies in prepucial secretions of naturally infected bulls. *Veterinary Pathology,* 36: 406-411.

Schonmann M J, BonDurant R H, Gardner I A, Hoosear K V, Baltzer W, Kachulis C and Van-Hoosear K. 1994. Comparison of sampling and culture methods for the diagnosis of Tritrichomonas infection in bulls. *Veterinary Records,* 134: 620-622.

Schwebke J R and Burgess D. 2004. Trichomoniasis. *Clinical Microbiology Reviews,* 17 (4): 794-803.

Soto P and Parma A E, 1989. The immune response in cattle infected with Tritrichomonas foetus. *Veterinary Parasitology,* 33: 343-348.

Soulsby E J L. 1982. Helminths, Arthropods and Protozoa of Domesticated Animals. Balliere Tindall, London, UK.

Skirrow S. 1987. Identification of trichomonad-carrier cows. *Journal of American Veterinary Medical Association.* 191: 553-554.

Skirrow S and BonDurant R H, Farley J and Correa J. 1985. Efficacy of ipronidazole against trichomoniasis in beef bulls. *Journal of America Veterinary Medical Association,* 187: 403-407.

Skirrow S and BonDurant R H, 1988. Bovine trichomoniasis. *Veterinary Bulletin,* 58: 591-603.

Skirrow S and BonDurant R H. 1990. Induced Tritrichomonas foetus infection in beef heifers, *Journal of American Veterinary Medical Association.* 196: 885-889.

Swai E S, Hulsebosch J and Vander Heijden W, 2005. Prevalence of genital campylobacteriosis and trichomoniasis in crossbred breeding bulls kept on zero-grazed smallholder dairy farms in the Tanga region of Tanzania. *Journal of South African Veterinary Medical Association,* 76 (4): 224-227.

Taylor M A, Marshall R N and Stack M. 1994. Morphological differentiation of Tritrichomonas foetus from other protozoa of the bovine reproductive tract. *Brazilian Veterinary Journal,* 150: 73-80.

Thomas M W and Harmon W M. 1994. Bovine Trichomoniasis: General Information: diagnosis and control. Visited November 18, 2010 from http://www.google.co.uk/imgres?imgurl=http://cati.csufresno.edu/sjer/rese/94/940301

Urquhart G M, Armour J, Duncan J L and Jennings F N, 2003. *Veterinary Parasitology.* Blacwell Publishers.

Wosu L U. 1977. Trichomonas infection in a bull – An apparent change in serotype of the infecting organism. *Australian Veterinary Journal,* 53: 340-341.

AN APPRAISAL OF THE USE OF VACCINATION FOR DISEASE PREVENTION IN POULTRY IN IBADAN, NIGERIA

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

Abstract

It has become almost practically impossible to engage in commercial poultry production without the challenges of diseases. Farmers therefore, have intensified efforts on various preventive measures including vaccination but with varying degree of success. This study was undertaken to assess the use and effectiveness of vaccination as a disease preventive measure in poultry. Structured questionnaires were administered to thirty poultry farmers randomly selected from Ibadan, Nigeria. Questions asked included those on the disease prevention strategies, source and types of vaccines, vaccination schedule and effectiveness of vaccination exercise in relation to frequency of disease outbreaks among others. Results showed that all the farmers practiced multiple preventive measures; 87% of them regularly vaccinate their chickens. Chickens were vaccinated against Marek's, Newcastle, Gumboro (IBDV), Coccidiosis, Fowl Pox, Fowl Typhoid, Infectious Coryza, Chronic respiratory disease and Fowl Cholera by 100%, 100%, 80%, 68%, 60%, 4%, 4%, 4% and 4% of the farmers respectively. From the 25 farmers that responded, 60% (15) used mainly imported vaccines while other farmers used local vaccines produced by the National Veterinary Research Institute, Vom, Nigeria. Vaccination still remains a very effective method of disease control since most of the farms that carried out vaccination reported no disease outbreaks.

Keywords: Vaccination, poultry diseases, preventive measures, vaccine failure

UNE EVALUATION DE L'UTILISATION DE LA VACCINATION COMME MESURE DE PREVENTION DES MALADIES DES VOLAILLES AIBADAN(NIGERIA)

Résumé

l'utilisation et l'efficacité de la vaccination comme mesure de prévention des maladies chez les volailles. Des questionnaires structurés ont été distribués à trente aviculteurs sélectionnés de façon aléatoire à Ibadan (Nigeria). Les questions posées portaient notamment sur les stratégies de prévention des maladies, la source et le type de vaccins, le calendrier de vaccination et l'efficacité des activités de vaccination par rapport à la fréquence des épidémies de maladies. Les résultats ont révélé que tous les aviculteurs mettaient en œuvre de multiples mesures de prévention ; et 87% d'entre eux vaccinaient régulièrement leurs oiseaux. Les volailles étaient vaccinées contre les maladies de Marek, de Newcastle, de Gumboro (IBDV), la coccidiose, la variole aviaire, la typhose aviaire, le coryza infectieux, les maladies respiratoires chroniques et le choléra aviaire respectivement par 100%, 80%, 68%, 60%, 4%, 4 %, 4% et 4% des aviculteurs.Des 25 aviculteurs ayant répondu au questionnaire, 60% (15) utilisaient essentiellement des vaccins importés, tandis que les autres utilisaient des vaccins produits localement par l'Institut national de recherche vétérinaire de Vom au Nigeria. La vaccination reste une méthode très efficace de contrôle des maladies, puisque la plupart des fermes qui la pratiquaient n'ont signalé aucune épidémie.

Mots-clés: Vaccination, maladies des volailles, mesures de prévention, échec vaccinal

Corresponding Author: olayinkaishola@yahoo.com

Introduction

Poultry production has undergone rapid changes during the past decades due to introduction of modern intensive systems of production, which places high demand on proper health care, hygiene and management but with small but very skilled labour force (FAO, 2000). Economic losses due to infectious diseases still remains the single major constraint hindering the success and growth of the poultry industry in Nigeria, where disease prevention and control measures are rare and high mortality rates are common even in vaccinated flocks in some cases (Ambali *et al.,* 2003). Disease prevention is best achieved by a combination of sanitation programmes and vaccination. Vaccination is one of the most important and cost-effective methods of preventing infectious diseases in animals (Lubroth *et al.,* 2007).

Vaccines are an important component of poultry disease prevention and control worldwide. A vaccine is any antigenic preparation administered with the object of stimulating the recipient's specific immune mechanisms in respect of given pathogen(s) or toxic agent(s) (Singleton and Sainsbury, 2006). They are also defined as disease-producing organisms, their parts or products which when introduced into animal or man are capable of evoking immune response which protects the vaccinated individual against infection by the micro-organism; they are products administered to animals in order to produce active or passive immunity or to diagnose the state of immunity (OIE, 2009). Their use in poultry production is traditionally aimed at avoiding or minimising the emergence of clinical disease at farm level and thus, increasing production (Marangon and Busani, 2006). Vaccines and vaccination programmes vary broadly in regard to several local factors (e.g. type of production, local pattern of disease, costs and potential losses) and are generally managed by the poultry industry. Vaccination against infectious diseases has been practiced for many decades and has proven the most cost effective means of alleviating animal suffering (Okolocha and Umoh, 2001) Vaccination has the advantages of stimulating active immunity, herd immunity and relatively cheap. In the last decade, the financial losses caused by the major epidemic diseases of poultry (avian influenza and Newcastle disease) have been enormous for both the commercial and the public sectors (Marangon and Busani, 2006).

This paper reports the findings of a survey of some poultry farms in Ibadan, South West Nigeria, to determine the use, source, availability and effectiveness of vaccination as a disease preventive measure in poultry.

Materials and Methods

Structured questionnaires were administered to thirty randomly selected poultry farmers located within Ibadan. The questionnaire focussed on the use, source, type, availability and storage of vaccines, diseases vaccinated against, personnel who carry out vaccination, types and effectiveness of disease preventive and control measures, frequency of vaccine failure, type of management in the farm among other issues raised. Also, verbal interview were conducted for some of the farmers to ensure proper understanding of some questions.

Data obtained were collated and analysed into descriptive statistics using Microsoft Excel. Pearson's correlation was used to determine the relationship between the factors studied (Shott, 1990).

Results

Vaccination of Birds against Diseases:

The study revealed all the 30 poultry farmers practiced various / multiple preventive measures including chemoprophylaxis, deworming, sanitation and hygiene. Twenty five (83%) of the farmers sampled regularly vaccinate their chickens as an additional disease preventive measure. Chickens were vaccinated against economically significant diseases such as Mareks, Newcastle, Gumboro (Infectious Bursal Disease, IBD), Coccidiosis, Fowl Pox, Fowl Typhoid, Infectious Coryza, Chronic Respiratory Disease (CRD) and Fowl Cholera by 100%, 100%, 80%, 68%, 60%, 4%, 4%, 4% and 4% of the farmers respectively (Fig. 1).

Types and Sources of Vaccines

Sixty percent (60%) of the farmers used only imported vaccines while the remaining used both imported vaccines and those manufactured locally by the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. Sixty percent (60%) of the farmers purchased their vaccines from veterinary drug shops in Ibadan while 40% purchased theirs from NVRI.

Personnel carrying out vaccination and effectiveness of the vaccination programmes

The study revealed that 15 farms (60%) have veterinary doctors carrying out vaccination programmes for the farm while in 16% of the farms, the owners/ farmers carry out the vaccination themselves. In the remaining farms, vaccinations were carried out by other farm workers (Fig. 2).

A total of 21 (84%) farmers amongst who were 15 veterinary doctors indicated that vaccination programmes carried out on their chickens were very effective (Table 1). A strong positive correlation which was statistically significant was established at +0.878 between the appropriateness of the personnel who carried out the vaccination and the effectiveness of the vaccination programme.

Availability and Storage of Vaccines

Eleven respondents (36.6%) indicated that vaccines used were regularly available; 8 (26.8%) indicated that they were irregular while other farmers declined comments. All the farmers that used vaccination as a disease preventive measure indicated the used of freezers, refrigerators, ice packs and sometimes cold rooms for storage of their vaccines.

Disease outbreak after vaccination

Only 3 (12%) farmers out of the 25 that vaccinated their flocks reported ever having disease outbreaks post-vaccination while the others reported no problem as long as their birds were vaccinated (Fig. 3). A strong negative correlation of -0.921 was found between effectiveness of vaccination programme and disease outbreaks post-vaccination.

Discussion

The current study showed that vaccination is commonly employed by poultry farmers in Ibadan as an effective disease preventive measure considering the high percentage (83%) of the farmers vaccinating their birds regularly and 88% that indicated that vaccination was an effective disease preventive or control tool. The use of vaccination as a tool for controlling poultry diseases has been recommended (Butchers and Miles, 2003). Vaccination should however, be used in combination with other management practices such as good husbandry, good sanitation and hygiene, chemotherapy and biosecurity programmes (Ambali *et al.,* 2003).

Diseases of high economic importance such as Newcastle, Mareks, Gumboro diseases and those of moderate economic significance were shown to be protected by vaccination. The results of this study differs from that of Ambali *et al.,* (2003), who reported scarcity of disease prevention and control measures by farmers in Nigeria and prevalence of high mortality rates in vaccinated flocks. The finding of 100%, 100% and 80% of the farmers that vaccinated against Newcastle, Mareks and Gumboro diseases respectively emphasises the importance attached to these diseases since they can cause mortality of up to 100% in infected flocks.

This study also confirms the preference of imported vaccines over locally manufactured NVRI vaccines; and fewer farmers obtained vaccines from NVRI. The reason for this could be due to irregular and inadequate supply and small doses of vaccines by NVRI. Most imported vaccines come in multiple higher doses and are also cheaper than NVRI vaccine. The consequences of relying heavily on imported vaccines include cost in foreign currency, unsteady availability of vaccines, transit and cold chain problems, uncoordinated variety of vaccine strains and programmes and increased losses through preventable mortality (Adene, 1997).

The strong positive correlation found between the appropriateness of the personnel who carried out the vaccination and the effectiveness of the vaccination programme

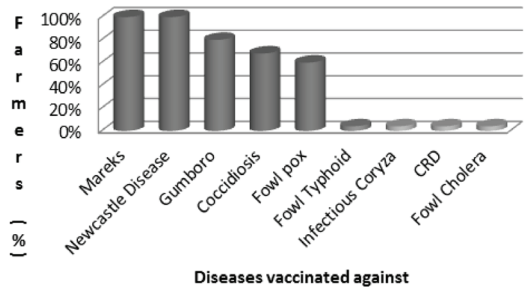

Fig. 1: Diseases commonly vaccinated against by Poultry Farmersa

Fig.2: Personnel carrying out vaccination programme

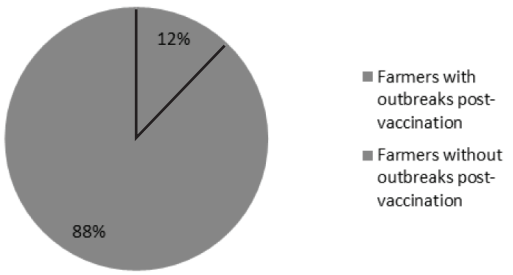

Fig.3: Proportion of farmers with disease outbreaks compared to those without outbreaks post-vaccination

Table 1: Effectiveness of vaccination programme

Effectiveness of vaccination program	Count (%)
Very effective	21 (84%)
Effective	4 (16%)
Not effective	0 (0%)
Total	**25 (100%)**

(with no disease outbreak) suggests that other categories of farm workers apart from qualified veterinarians should be discouraged from conducting vaccinations, as this may lead to vaccine failure. A vaccination failure occurs when, following vaccine administration, the chickens do not develop adequate antibody titre levels and/or are susceptible to a field disease outbreak (Butchers and Miles, 2003). When a vaccination fails, the natural inclination is to blame the vaccine. Although this is certainly an important consideration, there are other factors that must be evaluated to determine the cause of the failure. These factors include faulty administration of vaccines, difficulty in maintenance of cold chain, reversal to virulence of attenuated organism, stress, maternal antibody (Okeke and Lamorde, 1988; Butchers and Miles, 2003). The finding of high (84%) proportion of the farmers which agreed that vaccine is an effective means of disease control agrees with an earlier report that reiterated that since the introduction of vaccines in health management of animals and man, and despite their shortcomings, they have proved to be very effective tools for disease prevention over the years (Okolocha and Umor, 2001). The application of the different vaccination options should be adjusted in diverse conditions according to the local pattern of disease, the level of biosecurity practised in different types of poultry production systems, and the level of challenge for each type of poultry operation (Marangon, and Busani, 2006). Hence, poultry producers are encouraged to continue to employ vaccination along with other preventive measures as a means of reducing disease occurrence in their flocks. NVRI should make their vaccines more readily available and produce higher doses for easier administration to large flocks.

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References

Adene DF, 1997. Diseases of poultry in Nigeria: An overview of the problems and solutions. *Tropical Veterinarian,* 15 (3&4): 103-110.

Ambali AG, Abubakar MB and James TE, 2003. An assessment of poultry health problems in Maiduguri, Borno State, Nigeria. *Tropical Veterinarian,* 21(3): 138-145.

Butcher GD and Miles RD, 2003. Vaccination failure in poufltry: Factors to consider. VM82, Veterinary Medicine-Large Animal Clinical Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida Visited February 15, 2010, http://edis.ifas.ufl.edu.

FAO, 2000. Food and Agricultural Organisation of the United Nations, Rome, Italy. Statistical Database Visited May 25, 2009, http://www.fao.org

Lubroth J, Rweyemamu M, Viljoen G, Diallo A, Dungu B, and Amanfu W, 2007. Veterinary vaccines and their use in developing countries. *Rev. Sci. Tech. Off. Int. Epiz.* 26 (1): 179-201.

Marangon S, and Busani L, 2006. The use of vaccine in poultry production. *Rev. Sci. Tech. Off. Int. Epiz.* 26 (1): 265-274.

OIE, 2009. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. *A publication of World Organisation for Animal Health.* Visited January 28, 2006, http://www.oie.int/eng/normes/mmanual/2008/pdf/1.1.08_VACCINE_PRODUCTION.pdf

Okeke EN and Lamorde AG, 1988. Gumboro disease and its control in Nigeria. In viral diseases of animals in Africa. Eds., Williams AO, Masiga WN: CTA of ACP/EEC (Lome Convention), pp: 288-300.

Okolocha EC and Umoh JU, 2001. Vaccine development in veterinary medicine: a review. *Sokoto Journal of Veterinary Science,* 3(2): 1-8.

Shott S, 1990. Statistics for health professionals. W. B. Saunders Co. Philadelphia. pp. 212-226.

Singleton P and Sainsbury D, 2006. Dictionary of Microbiology. John Wiley and Sons Limited, London. 904p.

ISOLATION AND ANTIBIOTICS SUSCEPTIBILITY PATTERNS OF ESCHERICHIA COLI O157:H7 FROM SELECTED DAIRY HERDS IN NIGERIA

Amosun E A[1]*, Olatoye I O[2], Adetosoye I A[1].
[1]Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan, Nigeria.
[2]Department of Veterinary Preventive Medicine and Preventive Medicine, University of Ibadan, Ibadan, Nigeria.

Abstract.

This study determined the safety of milk from dairy herds obtained by hand milking method from two major cattle producing States of Nigeria by investigating the presence of Escherichia coli 0157:H7 and their antibiotic susceptibility pattern. Forty seven milk samples from Kwara and 63 from Kaduna States were obtained from selected indigenous breeds of dairy cow. Bacteriological analysis by culturing on MacConkey agar (MAC) and subcultured on Sorbitol MacConkey agar (SMAC) were done. Escherichia coli 0157.H7 were confirmed serologically using latex agglutination kits (OxoidR UK). The isolates were tested for susceptibility to five commonly used antimicrobial agents and plasmid transfer was also carried out using E. coli K12 356 recipient. Out of the 61 non-Sorbitol fermenting (NSF) E. coli isolated from the samples 33(30.0%) were confirmed as E .coli 0157.H7 serotype. Antibiotic Susceptibility profile showed that all the isolates were resistant to one or multiple antibiotics, resulting in six different resistance patterns. Sulphadimidine resistance was the highest with all the isolates (100%) exhibited resistance to this drug while streptomycin had the highest sensitivity. Out of the seventeen E .coli 0157.H7 isolates tested for plasmid transfer, eleven (64.7%) transferred their resistance to the sensitive E. coli K12 356 enblock, while the remaining six showed segregation. The preponderance of E. coli O157 in this study indicated that greater proportion of milk being produced for human consumption in Nigeria were not wholesome and could posed threat of transmission of zoonotic pathogens. The high proportion of multidrug resistance exhibited by the isolates and the plasmid transfer is of public health significance as they could confer resistance on both pathogenic and non-pathogenic bacterial population in the consumers. More so, the milk which were obtained traditional unhygienic hand milking are either consumed raw or improperly pasteurized. Hygienic milking, pasteurization and judicious use of antibiotics after properly diagnosis and sensitivity test against newer antibiotics are recommended.

Key words: EHEC, Escherichia coli, dairy, antibiotics, Nigeria

ISOLEMENT ET SENSIBILITÉ AUX ANTIBIOTIQUES DES BACTÉRIES ESCHERICHIA COLI O157:H7 (EHEC) CHEZ DES TROUPEAUX LAITIERS SÉLECTIONNÉS AU NIGERIA

Resume

La présente étude a examiné la sécurité sanitaire du lait obtenu par traite manuelle de troupeaux laitiers dans deux États du Nigeria (Kwara et Kaduna) qui sont de grands producteurs de bovins, en recherchant la présence des bactéries Escherichia coli 0157: H7 et leur sensibilité aux antibiotiques. Quarante-sept (47) échantillons de lait provenant de Kwara et 63 de Kaduna ont été obtenus de vaches laitières de race locales. Une analyse bactériologique par culture sur gélose de MacConkey (MAC) et repiquage sur gélose de MacConkey au Sorbitol (SMAC) a été effectuée. Les Escherichia coli 0157.H7 ont été confirmées sérologiquement en utilisant des kits d'agglutination au latex (OxoidR UK). Les isolats ont été testés pour vérifier leur sensibilité à cinq agents antimicrobiens couramment utilisés, et un transfert de plasmides a été également effectué en utilisant des E. coli K12 356 réceptrices. Des 61 E. coli ne fermentant pas le sorbitol (NSF) isolés à partir des échantillons, 33 (soit 30,0%) ont été confirmés comme E. Coli de sérotype 0157.H7. La sensibilité aux antibiotiques a montré que tous les isolats étaient résistants à un ou plusieurs antibiotiques ; et l'étude a révélé six profils de résistance différents. La résistance à la sulfadimidine avait le taux le plus élevé car tous les isolats (100%) présentaient une résistance à ce médicament, tandis

*Correspondence Author: elizabethamosun@yahoo.com

que la streptomycine avait la sensibilité la plus élevée. Des dix-sept isolats d'E .coli 0157.H7 testés pour le transfert de plasmides, onze (64,7%) ont transféré en bloc leurs résistances aux bactéries sensibles E. coli K12 356, tandis que les six autres ont montré une ségrégation. La prépondérance des bactéries E. coli O157 dans cette étude était une indication qu'une grande proportion de lait produit pour la consommation humaine au Nigeria n'était pas saine et pouvait poser une menace de transmission d'agents pathogènes zoonotiques. La forte proportion de multirésistance montrée par les isolats et le transfert de plasmides est significative pour la santé publique car la résistance pourrait être conférée à la fois aux populations bactériennes pathogènes et non pathogènes chez les consommateurs. Plus encore, le lait obtenu par traite manuelle traditionnelle et non hygiénique est soit consommé cru ou mal pasteurisé. En conséquence, la traite hygiénique, la pasteurisation et l'utilisation judicieuse des antibiotiques après un diagnostic adéquat et un test de sensibilité aux nouveaux antibiotiques sont recommandés.

Mots-clés: EHEC, Escherichia coli, laitier, antibiotiques, Nigeria

Introduction

Escherichia coli O157:H7 (EHEC) is one the six groups of pathogenic *E. coli* recognized as aetiological agents of diarrhoea (Aboaba, 2006). The major public health concern associated with the strain is the hemorrhagic colitis and production of shiga-like toxin (Griffin 1995). The organism was first isolated in 1982 (Riley *et al.,* 1983) and has since been associated with several food borne outbreaks around the world including Nigeria (Ojo *et al.,* 2009). The U.S. Centers for Disease Control and Prevention estimated that *E. coli* O157:H7 caused approximately 73,400 illnesses and 60 deaths each year in the United States (Mead *et al.,* 1999), while WHO, (2000) stated that, VTEC O157 isolation rates from cattle in the UK ranged from 0.9% to 15.7%. Aibinu *et al.,* (2007) reported 4.6% prevalence in animals and 1% prevalence in humans of E. coli O157:H7 infection in Nigeria.

The outbreaks were usually linked with the consumption of improperly handled or cooked foods of bovine origin, including beef and unpasteurized and contaminated post pasteurization dairy products (Karmali, 1989). Cattle has been implicated as the major reservoir of this organism, although there have been several reports of these organisms in sheep and other animals (Aibinu, *et al.,* 2007). They are also shed from the feaces of apparently healthy animals Tauxe, (1997), an increasing number of outbreaks of E. coli O157 associated illness have been attributed to water contamination and the consumption of raw vegetables possibly contaminated with bovine manure (Mead *et al.,* 1999). Carcasses can become contaminated through contact with intestinal contents at slaughter (Olatoye, 2010) while milk contamination could be due to endogenous infection or feacal contamination during milking and suckling. Ameh *et al.,* (1999) isolated *E. coli* with 6.7% prevalence from mastitis cows in Maiduguri, while Luga *et al.,* (2007) obtained a prevalence of 0.4% isolation of STEC O157 from water being consumed by dairy cattle in northwestern Nigeria. *E. coli* was also among the microorganisms isolated by Obi and Ikenebomeh, (2007) from raw and fermented milk in south-central states of Nigeria.

The hand milking operations employed by dairy farmers in Nigeria predisposes milk and milk products to contamination with various microorganisms including pathogenic E. coli. Furthermore, local processing (pasteurization) of milk is not usually effective at destroying contaminating microbes (Amosun *et al.,* 2009). More so, some portion of the milk is consumed raw by the milkmaid's households or public consumers. Hence this study investigated the incidence of EHEC in raw milk meant for consumption by milkmaids and their families to assess their risks of milk-borne communicable zoonoses.

Mastitis is considered the most economic important disease of dairy industry worldwide (Kossaibati and Esslemont, 1997) with economic losses and mortality (Hogan and Smith, 2003; Morin *et al.,* 1993; Stott and Kennedy, 1993). Coliform bacteria including *E. coli* are among the common pathogens responsible for environmental mastitis (Lam, 1996). Contamination of milk and milk products by various pathogenic bacteria results from poor

hygiene and milking operations especially the hand-milking practice by herdsmen in Nigeria (Amosun *et al.,* 2009). Notably, antibiotics are routinely and indiscriminately used by herdsmen for treatment of cattle in Nigeria. Such use is considered to be a major risk factor for development of antibiotic resistance among the pathogens (Witte, 1998; Olatoye, 2010) and may lead to antibiotic residues in milk. Hence, because of the potential zoonotic risk, we investigated the incidence of EHEC in raw milk meant for consumption by milkmaids and their families in Nigeria.

Materials and Methods

Study Area and sample collection

Kaduna State is located in the north central Nigeria while Kwara State is located in the central Nigeria, where the majority of cattle are reared. Forty seven raw milk samples from Kwara and 63 from Kaduna were obtained from selected indigenous dairy cow in the two states in Nigeria. The udders were washed in disinfectant, the initial stream of fore-milk was discarded after which about 5ml of milk from each cow was collected respectively into a sterile bottle aseptically.

Immediately after collection, sterile swabs were used to obtain milk from each sample bottle and inoculated into sterile Tryptone Soy broth (TSB). These cultures were transported on ice (Coleman® Flask) to the Department of Veterinary Microbiology and Parasitology, University of Ibadan. The broth cultures were incubated at 37^{0}C for 18hrs after which a loop full was inoculated on to MacConkey agar (MAC) and Blood agar plates. All colonies that were lactose fermenters on MacConkey agar, Gram-negative rod, motile, catalase negative, oxidase negative, indole positive and therefore showed the standard biochemical characteristics of *E. coli* described by Barrow and Felthman (1993), were sub-cultured on Sorbitol MacConkey (SMAC Oxoid CM 813, UK) agar plates, and incubated at 37OC at 24 hr. Colonies that were colourless to pale, flat and smooth, circular or serrated at the edge were selected for further testing as presumptive non-sorbitol fermenting E. coli. All non-sorbitol fermenting E. coli strains were tested using latex agglutination kit for E. coli 0157:H7 (Oxoid DRO 120M, UK) according to the manufacturer's instruction. The minimum inhibitory concentrations of *neomycin, streptomycin, tetracycline, sulphadimidine* and *ampicillin* (sourced from Sigma Aldrich Chemical Ltd, USA) were determined as described by Rollins *et al.,* (2003). The antibiotics were respectively dissolved in sterile Tryptone soy broth (TSB) to a final concentration of 20µg/ml. *Escherichia coli* ATTCC25922 were used as control organism. Escherichia coli 0157:H7 that showed resistance to the antibiotics but sensitive to streptomycin were used for plasmid transfer study with Escherichia coli 356 K12.

Serological analysis

All non-sorbitol fermenting strains were tested for the presence of 0157:H7 with latex agglutination kit for E. coli 0157:H7 (Oxoid DRO 120M, UK) according to the manufacturer's instruction. A drop of saline was added to the small ring in both tests and control reaction areas. A portion of suspected colony was picked with a sterile stick and carefully emulsified in a saline drop. The mixing sticks from the kit were used to spread the mixture over the entire area of the ring the same was done with the control latex and control organism. The card was then rocked after which visible agglutination within one min was considered positive.

Plasmid transfer

Donor strains: Seventeen Escherichia coli 0157:H7 isolates that showed resistance to sulphadimidine; neomycin, ampicillin and tetracycline were used in this investigation.

Sensitive recipients: Escherichia coli 356 K12 resistant to 200ug /ml streptomycin was used as recipient while the donor strain was sensitive to streptomycin.

Selective media

The selective media were prepared by addition of 50 µg/ml of streptomycin and 25µg/ml each of the antibiotics studied namely, tetracycline, ampicillin, neomycin and sulphaldimidine into 25ml of MacConkey agar.

Conjugation procedure

Seventeen E. coli 0157: H7 that were multidrug resistant but sensitive to streptomycin at MIC ≤16 µg/ml were used respectively as donor in the resistant plasmid transfer studies. Conjugation procedure reported by Walton (1972) and modified by Adetosoye (1980) was employed in this study.A discrete colony of each resistant *E.coli* 0157:H7 as well as *E.coli* 356 K12 was respectfully inoculated into 10 ml of sterile Tryptone Soy broth (TSB) contained in sterile test tubes with cork. The cultures were incubated aerobically at 37^{0}C for 10h. Subsequently 0.02ml of the donor culture was delivered into sterile 10 ml sterile Tryptone Soy broth (TSB) and 0.04ml of the recipient *E.coli* K12 was added the mixture was similarly incubated aerobically at 37^{0}C for 18h. The transconjugants were incubated onto sterile MacConkey agar (Oxoid CM 109R) plates containing mixture of different concentration of antibiotics formulation. 50 µg/ml of streptomycin was added to 25ml of MacConkey agar the plates and each of the plates was subsequently incorporated with 25µg/ml each of the antibiotics studied shown in table 3. The plates were incubated at 37^{0}C overnight. All the transconjugants plates with growth on the selective media were considered as having acquired resistance from the donor strains.

Antibiotic sensitivity of the transconjugants

In-vitro antibiotic sensitivity test of each of the transconjugants was carried as described by Walton (1972) and modified by Adetosoye (1980). A colony each of the transconjugants was inoculated into 5ml sterile Tryptone Soy broth (TSB) and incubated at 37^{0}C for 8h.The 0.01 ml portion of the culture was delivered into 4 ml of sterile Tryptone Soy broth (TSB) and the mixture was vigorously shaken to give a 1:2000 dilution. Subsequently nutrient agar plates (for sensitivity test) were inoculated by flooding with the 1:2000 diluted broths. The excess broth was drained off and the plates were allowed to stand on the bench for 1h after which antibiotic discs of tetracycline (60µg), ampicillin (60µg), and neomycin (60µg), sulphaldimidine (50µg) were aseptically and respective applied. The test plates were allowed to stand on the bench for 1h to allow the antimicrobial agents to diffuse into the agar. The plates were then incubated at 37^{0}C for 18h after which the results were recorded as resistance transfer either by segregation or enbloc.

Results

A total of 33 E. coli O157:H7 isolates were obtained from milk from Ilorin and Kaduna and isolation rates differentiated between states (Table 1). All isolates were confirmed by serotyping as E. coli O157:H7 also haemolysed sheep red blood cells. The percentages resistance of the isolates to common used antibiotics include sulphadimidine (100%) followed by tetracycline 27(81.8%), neomycin 18(54.5%) , ampicillin 17(51.5%) and streptomycin 15 (45.5%) as shown in figure 1. The 17 E. coli O157:H7 strains used for plasmid transfer study had one or multiple resistance patterns (Table 2), but were all sensitive to streptomycin. Eleven of seventeen (64.7%) transferred R- factor for tetracycline, ampicillin, neomycin, sulphadimidine and streptomycin; three (17.6%) of the isolates transferred for tetracycline, ampicillin, sulphadimidine; one (5.88%) of the isolates transferred for tetracycline, neomycin, sulphadimidine; another one (5.88%) transferred for tetracycline and neomycin; and the remaining one (5.88%) transferred for tetracycline.

Discussion

Cattle have been reported to be the principal reservoir of E. coli 0157:H7 (WHO, 1998). The presence of the organism in faeces of cattle constitutes a potential source of spread of the pathogen and contamination of meat and milk from the animal. The organisms could also be isolated from milk of apparently healthy cow (Obi and Ikenebomeh, 2007). In the present study we obtained a prevalence rate of 55.45 % of the *E. coli* 0157:H7 from the milk of health cow. This finding is higher than 13.1% prevalence of *E. coli* 0157.H7 in dairy cattle reported by Low *et al.,* (2005) in Scotland.

Table 1: E.coli 0157:H7 isolated from raw milk samples in Nigeria.

Location	No of samples obtained	No of NSF* E.coli isolated	No of E. coli 0157 isolated
Kaduna (Kaduna State)	63 (57.27%)	33 (52.38%)	23 (36.51%)
Ilorin (Kwara State)	47 (42.73%)	28 (59.57%)	10 (21.27%)
Total	**110**	**61 (55.45%)**	**33 (30.0%)**

*NSF non-sorbitol fermenter

Table 2: Result of Antibiotic Resistance transfer mode by Trasconjugant of the E.coli 0157:H7

Antibiotic transfer	No of strains showing profile	Form of transfer	Location
Tet	1	segregation	Ilorin
Tet, Neo	1	segregation	Kaduna
Tet, Amp, Sulph	3	segregation	Kaduna=1 Ilorin=2
Tet, Neo, Sulpha	1	segregation	Kaduna
Tet, Amp, Sulpha, Neo.	11	Enbloc	Kaduna=7 Ilorin=4

This finding is of food safety concern as the organism could also contaminate the bulk milk obtained from the same herds during the unhygienic hand milking methods usually employed by the herdsmen and milkmaid (Amosun *et al.,* 2009). The higher value obtained in the present study could be due to the prevailing management system that constitutes stress for the animal. They are reared on semi-intensive systems where they trekked long distances for grazing and water. These stress factors, as well as lactation and milking stress could affect the immune system and lower the resistance of the cow resulting in the multiplication of the organism in the gut and consequently increasing the chances of its dissemination in to the mammary glands of such animal thereby increasing the shedding of *E. coli* 0157.H7 in the milk. Moreover, congregation of cattle brought from various sources to a dairy herd could be another factor responsible for the higher rate of STEC 0157.H7 recovering from different dairy cows at the individual farms.

It is possible that animals originally were free from the organisms before leaving the farm. They could get it either from the already contaminated environment through penetration into udder through a cut or wound on the mammary glands of such animals or from carrier animals from other farms actively shedding it. This will equally result in a higher prevalence rate of the STEC 0157:H7 be shed in individual farm animals on various farms in the community.

This study confirmed the presence of STEC 0157.H7 in dairy cattle, which constitutes potential threat to public health. It was first identified as a cause of illness in 1982 (Riley *et al.,* 1983) and the infections have now been reported with increasing frequency (Fitzpatrick, 1999). A multi-state large outbreak of food poisoning in Washington, Idaho, California and Nevada was traced to a common source of hamburger from a fast food restaurant (Koneman *et al.,* 1997). Vegetable and other foods have also been reported to be contaminated by STEC O157 from faeces of cattle leading to outbreak of food poisoning (Zschock *et al.,* 2000; Kuhnert *et al.,* 2005). The source of most cases of *E. coli* related illness in the United States is considered to be source of food from cattle origin (Koneman *et al.,* 1997). The results observed in this study and other studies (Khan *et al.,* 2002; Anon, 2005; Kuhnert *et al.,* 2005) suggest a pattern describing the level of risk of exposure to STEC 0157. It seems everyone involved in cattle-related enterprise is at a potential risk of exposure. Kuhnert *et al.,* (2005) reported that *E. coli* 0157. H7 was present in 25% of farms with organic production while incidence in farms with interacted (conventional) production was 17%. Another study reported that 38.5% of dairy

Table 3: Reconstitution with different concentration of Antimicrobial agents (µg/ml) of MacConkey agar for use as selective media.

S/N	Medium	Tet	Neo	Strept	Amp	Sulpha
1	A	-	-	50		
2	B	25	-	50	-	-
3	C	-	25	50	-	-
4	D	-	-	50	25	-
5	E	-	-	50	-	25

Table 4: Antibiotics resistance pattern of E.coli 0157:H7 used for plasmid transfer

Antimicrobial agents	No of strains showing resistance	Location
Su	4	Kaduna
PNSu	2	Ilorin
TeSu	1	Kaduna
TeSuN	11	Kaduna
PNTeSu	8	Ilorin
PNTeSuN	7	Kaduna

Su=Sulphadimidine, PN=Ampicillin, Te =tetracycline, N=Neomycin

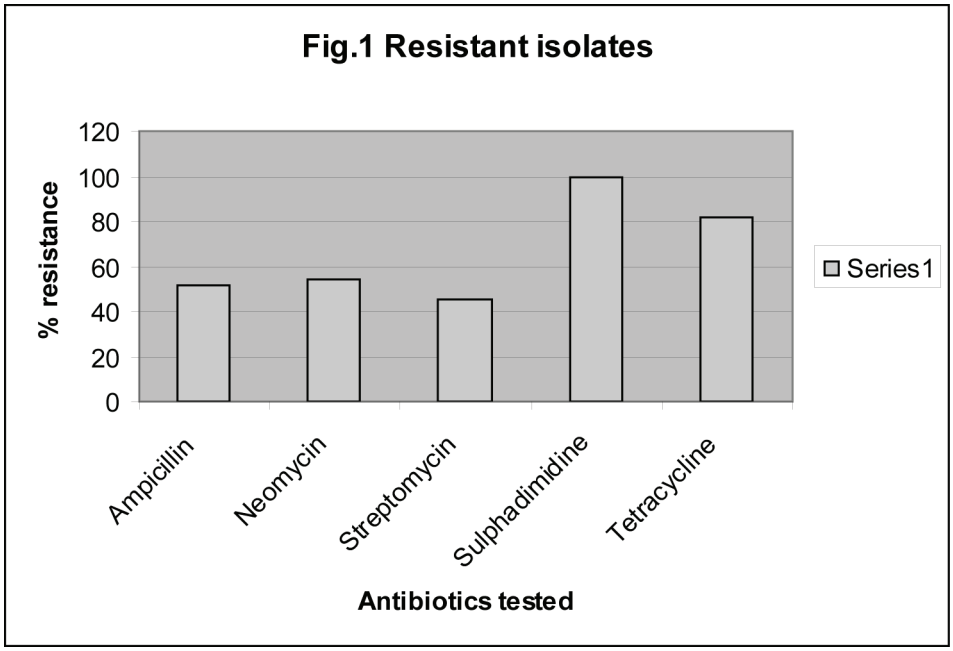

Fig 1: Percentages of E.coli 0157:H7 isolates showing resistance to common used antibiotics.

farms have at least one cow that was positive for *E. coli* 0157.H7 but only 4.3% of individual cows were shedding the pathogen (Anon, 2005). It can be inferred that the incidence of *E. coli* 0157.H7 is higher when cattle are conveyed from one farm to another. This study confirmed the presence of E. coli 0157.H7 in the indigenous dairy cattle production in Nigeria, thereby posing a potential danger of outbreak of milk-borne *E. coli* 0157.H7 infection among consumers on improperly pasteurized or raw milk and in people engaged in cattle related activities.

The isolates obtained in this study also showed multi-drug resistance to the commonly used antibiotics both for the prevention and

control of bacterial infections including mastitis. The high prevalence of multi-drug resistance of E. coli 0157.H7 to antibiotics is calls for concern. The indiscriminate use of antibiotics in livestock enterprises and by unauthorized individuals must be discouraged. Therefore the use of antibiotics in Nigerian livestock production should be properly regulated so that it can only be used under the prescription and supervision of a certified veterinarian and other trained medical personnel.

Reference:

Reference

Adetosoye A I: Transmissible drug resistance in human and animal strains of Enterobacteriaceae and Pseudomonas aeruginosa. *Res. Vet. Sci.* 29: 342-345 (1980).

Aibinu I E, Peters F R, Amisu K O, Adesola S A, Ojo M O, Tolu O, 2007. Multi drug resistance in E coli 0157 strains and the public health implication. *Journal of American Science*, 3(3): 22-33.

Ameh J A, Egbe-Nwiyi T, Zaria L T, 1999. Prevalence of bovine mastitis in Maiduguri Borno State, Nigeria. Veterinary Archives, 69:87-95.

Amosun E, Olatoye O I, Adetosoye A I, 2009. The prevalence of gram negative bacteria in bovine mastitis among dairy herds in Nigeria. *Tropical Veteterinarian*, 27(3): 25-29.

Anonymous 2005. E. coli 0157.H7 and Salmonella typhimurium DT 104: The role of animals.; http://www.vetmed.wisc.edu/pub/zoonoses/0157/DT104/0157DT104index.html

Barrow G T, Feltham R K A, 2005. Cowan and Steel's Manual for the Identification of medical bacteria, 3rd ed. *Cambridge University Press*, Cambridge.

Fitzpatrick M, 1999. Haemolytic uraemia syndrome and E. coli 0157. Prevention rests with sound public health measures. Br. *Med. J.* 318:684-685.

Griffin P M, 1995. Escherichia coli 0157:H7 and other hemorrhagic Escherichia coli. In Infection of the Gastrointestinal Tract, Eds., BlaserMJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RI: Raven press, Ltd. New York pp: 739-761.

Hogan J S, Smith K L. 2003: Coliform mastitis (Review article). Vet. Res. 34: 507-519.

Karmali M A, 1989. Infection by verocytotoxin-producing Escherichia coli. Clin Microbiol Rev. 2: 15-38.

Khan A, Yamasaki S, Sato T, Ramamurthy T, Pal A, Datta S, Chowdhury N R, Das S C, Sikdar A, Tsukamoto T, Bhaltacharya S K, Takeda Y, Nair GB, 2002. Prevalence and genetic profiling of virulence determinants of non 0157 shiga toxin-producing E. coli isolated from cattle, beef and human, calcultta. India, Emerg. *Infect. Dis*. 8 (1):54-62.

Koneman W E, Allen D S, Janda M W, Schrechkenberger C P, Winn C W, 1997. Color atlas and textbook of diagnostic microbiology. 5th Ed. Lippincott Williams & Wilkins.

Kossaibati M A, Esslemont R J, 1997. The costs of production diseases in dairy herds in England. Veterinary Journal, 154:41-51.

Kuhnert P, Dubosson C R, Roesch M, Homfeld E, Doherr M G, Blum J W, 2005. Prevalence and rik factor analysis of Shiga toxigenic E. coli in faecal samples of organically and conventionally farmed dairy cattle. *Vet Microbiol.* SS 109:37-45.

Lam T J G M, 1996. Dynamics of bovine mastitis, PhD thesis, Utrecht University.

Low J C, McKendrick, I J, Mckenchnie C, Fenlon D, Naylor SW, Currie C, Smith DGE, Allison L, Gally DL, 2005. Rectal carriage of enterohaemorrhagic E. coli 0157 in slaughtered cattle. Appl. and Environ. Microbiol.; 71(1):93-97.

Luga I I, Akodu I, Mhomga I, Allan L, Ajogi I, Umoh V J, Kwaga J K P, 2007. Antimicrobial resistance of shiga toxin producing E.coli 0157.H70157 NM isolates from water fed to cattle in northwestern, Nigeria. Asian J. Anim. Vet. Ad.; 2(4):205-211.

Mead P S, Slutsker L, Dietz V, McCaig L F, Bresee J S, Shapiro C, Griffin P M, Tauxe R V, 1999. Food-Related Illness and Death in the United States. *Emerging Infectious Diseases* 5(5):607-25.

Morin D E, Peterson G C, Whitmore H L, Hungerford LL, Hinton RA, 1993. Economic analysis of a mastitis monitoring and control program in four dairy herds. *J Americ Vet Med.* Ass. 202(4): 540-548.

Obi C N, Ikenebomeh M J, 2007. Studies on the microbiology and nutritional qualities a Nigerian fermented milk product nono. *Int J Dairy Sc.* 2(1):95-

99.

Ojo O E, Oyekunle M A, Ogunleye A O, Otesile E B. .E.coli 0157:H7 Prevalence and in vitro antimicrobial susceptibility in food animal in part of Southwestern Nigeria. *Trop. Vet*. 2009; 26: 23-30

Olatoye I O, 2010. The incidence and antibiotics susceptibility of Escherichia coli 0157:H7 from beef in Ibadan Municipal, Nigeria. *Afr. Jour Biotech*. 9:1196-1199

Riley L W, Remis R S, Helgersons D, 1983. Haemorhagic colitis associated with rare E. coli serotypes. *N Engl J. Med*. 308: 681-685.

Rolllins D M, Temenak J J, Shield P, Joseph S W, 2003. *Microbial Pathogeesis Laboratory Manual. 2nd Edition.*

Stott A W, Kennedy J O, 1993. The economics of culling dairy cows with clinical mastitis .*Vet Res.*; 133 (20): 494-498.

Tauxe J D, 1999. A Probabilistic Physical System Model of Multipathway Contaminant Transport for Assessment of Future Risk. Fall Meeting of the American Geophysisical Union, San Francisco, California. 13-17.

Walton J R, 1972. A dynamic study of drug resistance in population of faecal E. coli from pigs fed nitrovin and furazoli done continuously. Zbl. Vet. Med. B 19:646-654.

WHO, 2000. Communicable disease, Rollins DM, Temenak.

World Health Organization, 1998. Zoonotic non 0157 Shiga toxin-producing E. coli (STEC). Report of a WHO scientific working group meeting. WHO/CSR/APH/98.8 http://www.who.int/emc.

Witte W, 1998. Antibiotic use in animal husbandry and resistance development in human infections. *APUA Newsletter*. 16 (3): 1, 4-6.

Zschock M, Hamann H P, Kloppert B, Wolter W, 2000. Shiga toxin-producing E. coli in faeces of healthy dairy cows, sheep and goats: Prevalence and virulent properties. *Let Appl. Microbiol* 31:203-208.

BACTERIA ASSOCIATED WITH SHEEP PNEUMONIA IN El - DAMAZIN AREA, THE BLUE NILE STATE, THE SUDAN

Sabiel Y A[1], Musa M T[2] and Hadia El- Jack Ahmed[2]

[1]Department of Bacteriology, Central Veterinary Research Laboratories, P.O. Box 8067 (Al-Amarat) Khartoum, Sudan. E. mail: sabiel-sd@hotmail.co.uk

[2]Animal Resources Research Corporation (Al-Amarat) Khartoum, Sudan Khartoum, Sudan, E. mail: musatibin@yahoo.com

Summary

Little work have been done in the Sudan concerning the association of different bacteria with sheep pneumonia. This study was conducted to isolate and characterize the aerobic bacteria associated with pneumonia in sheep by conventional bacteriological methods. A total of 250 samples were collected from lungs of sheep with pathological conditions at El –Damazin Slaughterhouse. The pathological lesions included: congestion, fibrinous inflammation, pleural adhesions and abscesses. The isolates were identified by the standard bacteriological methods. Two hundred and two organisms were isolated from the 250 samples of which 100 (49.5%) were Gram- positive and 102(50.5%) Gram-negative bacteria. The gram-negative bacteria isolated were 20(10.9%) Mannheimia haemolytica, 15(7.6%) Pasteurella multocida, 13(6.4%) Citrobacter, 11(5.4%) Klebsiella spp., 10(5.0%) Actinobacillus ligneirsii, 9(4.5%) Pseudomonas aeruginosa, 7(3.4%) Histophillus somni, (3.4%) Escherichia coli, 5(2.5%) Enterobacter spp. and 5(2.5%) Bordetella parapertussis. The Gram-positive isolates were 45(22.5%) Staphylococcus spp. 30 (14.9%) Streptococcus and Enterococcus spp. 17(8.8%) Corynebacterium pseudotuberculosis and 8(4.0%) Arcanobacterium (Actinomyces) pyogene.

Keywords: Pneumonia, sheep, Pasteurella, Mannheimia, conventional tests

LES BACTERIES ASSOCIEEA LA PNEUMONIE DES MOUTONS DANS LA REGION D,EL-DAMAZIN, ETAT DU NIL BLEU, SOUDAN.

Résumé

Peu de travaux ont été menés au Soudan portant sur l'association de différentes bactéries à la pneumonie des moutons. La présente étude a été faite dans le but d'isoler et caractériser les bactéries aérobies associées à la pneumonie chez les moutons par des méthodes conventionnelles bactériologiques. Un ensemble de 250 échantillons a été recueilli à partir de poumons des moutons présentant des conditions pathologiques à l'abattage d'El-Damazin. Les lésions pathologiques inclus : la congestion, les fibrineux, l'inflammation, les adhérences pleurales et les abcès. Les isolats ont été identifiés par la méthode bactériologique standard. Deux cent deux organismes ont été isolés à partir de 250 échantillons dont 100(49.5%) étaient à Gram-positif et 102(50.5%) bactéries Gram-négatives. Les bactéries gram-négatives isolées étaient 20(10.9%) Mannheimia haemolytica. 15(7.6%) Pasteurella multocida, 13(6.4%) Cirobacter, 11(5.4%) Klebsiella spp., 10(5.0%) Actinobacillus ligneirsii, 9(4.5%) Pseudomonas aeruginosa, 7(3.4%) Histophillus somni, (3.4%) Escherichia coli, 5(2.5%) Enterobacter spp. et 5(2.5%) Bordetella parapertussis. Les Gram-positifs isolats étaient 45(22.5%) Staphylococcus spp. 30(14.9%) Streptococcus et Enterococcus spp. 17(8.8%) Corynebacterium pseudotuberculosis et 8(4.0%) Arcanobacterium (Actinomyces) pyogene.

Mots clés: Pneumonie, moutons, Pasteurella, Mannheimia, tests conventionnels

Correspoding author: sabiel-sd@hotmail.co.uk

Introduction

Livestock is an important contributor to the national economy and plays an essential role in the traditions and cultures of Sudanese people. Before oil was exploited in the country, livestock and its products were the country´s most important revenue for foreign exchange, but currently it ranks the second after oil (el-Dirani *et al.,* 2009). This study was conducted to investigate into causes of sheep pneumonia in El-Damazin area following complaints of animal owners. Diseases affecting the respiratory system are generally the most important in domestic animals. In sheep they are more frequent and complicated compared to other domestic animals because the ratio of the alveolar surface to the metabolic weight is low (Casamitijana,1994). A single agent may start the infection and when the local resistance of the respiratory mucosa is lowered, bacteria inhabitant in the nose and the pharynx extend downwards resulting in multiple bacterial infections. Pneumonia in sheep causes loss of weight gain and increased predisposition to pleurisy resulting in significant losses due to reduction of lamb´s growth rate and increases mortalities of neonatal and adult sheep (Cathryne, 2006). The aetiology of pneumonia in sheep is complex, usually combination of agents and stress factors that weaken sheep´s immune system by triggering increased secretion of hormones from the endocrine system (Jones *et al.,* 1997). The agents are mainly bacteria like Mannheimia, Pasteurella, Mycoplasma, Klebsiella, Bordetella, Haemophillus, Escherichia coli, Staphylococcus, Streptococcus, Corynebacterium and Chlamydia spp. or other agents like viruses such as Para Influenza v type 3, Respiratory cyncytial, Bovine herpes type 1, Bovine viral diarrhoea, Blue tongue and Contagious ecthyma viruses (Lehmkuhl *et al.,* 2007). Mannheimia haemolytica (M. haemolytica) is considered to be the main causative agent of pneumonic pasteurellosis, although many investigators still believe that Pasteurella multocida (P.multocida) also plays a role in the disease (Quinn *et al.,* 2001). M. haemolytica and P. multocida were isolated frequently from nasopharynx of healthy and sick animals (Shigidi, 1976; El-Sanousi *et al.,* 1991; Biberstein *et al.,* 1999). Many investigators reported the a association of other bacteria with sheep pneumonia (Rhaymah *et al.,* 2001; Alton *et al.,* 2006; Yimer and Asseged, 2007; El-Sanousi and Tag El-din, 1971).

Materials and Methods

A total of 250 samples from pneumonic lungs of sheep were collected at AL- Damazin Slaughter House. The lesions included 64(25.6%) congestion, 84(33.6%) fibrinous inflammation, 61(24.4%) pleural adhesion, 31(12.4%) Mucopurulent inflammation and 10(4.0%) abscesses. Samples were collected from the edges of the lesions including healthy tissues. Sterile surgical scalpel blades and forceps were used for cutting and removal of sections of the affected lungs. Each sample was placed in a sterile plastic bag and transported in a thermosflask with ice to El- Damazin Regional Veterinary Research Laboratory and cultured on the same day. Isolation was attempted from the samples by deep incisions on the surface of each sample with a sterile scalpel. A sterile swab was dipped into each incised area and streaked onto 5% sheep blood agar plate. The plates were incubated aerobically at 37˚C for 24 h. If no growth was observed, the incubation was continued for 48 h before the plates were discarded as negative .All the samples were then stored at -20˚C in a deep freezer after they were cultured. The pure bacteria were grouped according to their Gram´s reaction and morphology and each was examined with the primary and the secondary bacteriological and biochemical tests for characterization (Quinn *et al.,* 1994; Barrow and Feltham, 1993; Smith, 1985)..

Results

Of the 250 samples 240 (96.0%) were positive for different bacteria of which 100 (49.5 %) were Gram-positive, 102 (50.5%), were Gram- negative and 40 (16%) Micrococcus species which were non significant. The bacteria isolated are presented in Table 1. The M. haemolytica strains were isolated in pure forms or mixed with other bacteria Their colonies on sheep blood agar, incubated at

Table 1: Bacteria isolated from sheep pneumonic lungs in El Damazin area.

No.	Isolates	Pure	mixed	total	percentages
1	Staphylococcus aurous	7	16	23	11.4%
2	Mannheimia haemolytica	6	14	20	10 %
3	Corynebacterium pseudotuberculosis	8	9	17	8.3%
4	Pasteurella multocida	3	12	15	7.6%
5	Citrobacter	10	3	13	6.4%
6	Streptococcus equi subs. zooepidermidis	8	4	12	6.0%
7	Klebsiella spp.	8	3	11	5.4%
8	Actinobacillus ligneirsii	7	3	10	5.0
9	Pseudomonas aeruginosa	4	5	9	4.5%
10	Staph. epidermidis	2	7	9	4.5%
11	Arcanobacterium (Actinomyces pyogene	4	4	8	4.0%
12	Histophillus somni	3	4	7	3.4%
13	Escherichia coli	3	4	7	3.4%
14	Streptococcus equi subs. equi	4	2	6	2.9%
15	Enterococcus faecalis	5	1	6	2.9%
16	Enterococcus faecium	5	1	6	2.9
17	Bordetelle Parapertussis	2	3	5	2.5%
18	Enterobacter	4	1	5	2.5%
19	Staphylococcus lentus	-	4	4	2.0
20	Staphylococcus intermedicus	2	1	3	1.4%
21	Staphylococcus hyicus	-	2	2	1.0%
22	Staphylococcus saprophyticus	-	2	2	1.0%
23	Staphylococcus zylosis	-	1	1	0.5%
24	Staphylococcus stomatis	1	-	1	0.5%
Total		**96**	**106**	**202**	**100%**

37^{0}C for 24 hours, were grey glistening, large, shiny and convex with irregular margins and had brown centres. They had a distinct smell, and clear zones of β –haemolysis beneath. The organisms produced pin point pinkish colonies on MacConkey agar. They were catalase and oxidase positive, reduced nitrate to nitrite, hydrolysed gelatine, phosphatase positive, produced no gas from glucose and negative for arginine, Vogus Proskaur and indole tests. All strains produced acid from maltose, mannitol, melibiose, sorbitol, sucrose and xylose but failed to ferment dulcitol, rhamnose, trehalose and celliobiose.

The P. multocida isolates were in pure cultures or mixed with other bacteria. They were Gram-negative occurring as short rods and coccobacilli, aerobic and facultative anaerobic. Colonies of the organism on blood agar were non- haemolytic, rounded, intermediate in size and sometimes small, gray and glistening. They did not grow on MacConkey agar, were non-motile, oxidase, catalase, nitrate and indole positive, and urease negative. They produced acid from: mannitol, sucrose, maltose, xylose, and did not produced acid from raffinose and salicin, 12 isolates (80%) were trehalose positive.

Colonies of Actinobacillus ligneirsii on a horse blood agar, incubated at 37°C for 24 hours, appeared flat, soft and glistening, and no growth was observed on MacConkey agar. They were Gram-negative rods and none-motile. They were positive for catalase, oxidase, nitrate, phosphatase, urease and aesculin tests, and negative for Potassium cyanide (KCN),

gelatine liquefaction, and indole tests. They did not produce gas from glucose and acid was produced from maltose, mannitol, sucrose, D-xylose, and no gas was produced from fermentation of L- arabinose, cellibiose, dulcitol, inositol, mellibiose, raffinose and trehalose. Isolates of Histophillus somni were non-haemolytic, small dewdrop-like colonies were developed when the organisms were inoculated onto chocolate agar, incubated in 10% Co2 (candle jar) at 37˚C for 24 to 48 hours. The organisms were motile by direct and hanging drop methods. H. somni was Gram-negative rods and sometimes coccobacilli. They were positive in oxidase, indole (weak), nitrate, and negative in catalase, urease, Vogus Proskaur and aesculin hydrolysis tests. No gas was produced in glucose and acid was not produced from mannitol, D- mannose, D- sorbitol, D-xylose, trehalose, and variable results were obtained in fermentation of L- arabinose and dulcitol. The other bacteria isolated and identified had typical characteristics described by the different authors (Quinn et al., 1994; Barrow and Feltham, 1993; Smith, 1985).

Discussion

In the Sudan evidence about the association of bacteria with pneumonia in sheep was earlier reported by some investigators. An organism resembling Actinobacillus actinoides was isolated from pneumonic lungs of a dead sheep (El-Sanousi and Tag El-din. 1971). In another study, 120 (30%) of the isolates, from nasopharynx of sheep were found to be P. haemolytica (Shigidi, 1976). P. multocida and Actinobacillus ligneirsii were isolated from dead and sick sheep in El-Hoda farm in the Sudan (El-Sanousi et al., 1978) . In later studies Staphylococcus aurous, Streptococcus pyogene, Corynebacterium pseudotuberculosis, Actinomyces spp., P. haemolytica and P. multocida were found to be associated with sheep pneumonia (Manal, 1998; Fadia, 1998). Their findings were in agreement to the results of this study and those of other investigators (Tabo, 2001; Zaitoun, 2001). The association of Actinobacillus ligneirsii, Histophillus somni, Klebsiella, E .coli, Citrobacter, Enterobacter, Pseudomonas aeruginosa and Bordetella parapertussis in pneumonia in sheep as shown in the present study was in agreement with previous reports (Ozbey and Muz. 2004; VIEN, 2008; Sayed, 1997; Ertan, 2006; Porter et al., 1994). Involvement of Staphylococcus, Streptococcus and Enterococcus in pneumonia in sheep were also reported by other investigators (Ozbey and Muz . 2004; Al-Sultan, 1995). The association of Corynebacterium pseudotuberculosis and Arcanobacterium pyogene in pneumonic condition in sheep as noted in the present study, was also reported by other investigators (Yimer and Asseged, 2007; Cynthia and Scott, 2005).

Conclusion

Pneumonia remains the most important diseases of livestock and the presence of the bacteria encountered in the upper respiratory tract of healthy sheep resulted in descending infections. Control of the disease may be achieved by correction of the predisposing factors, vaccination specially against pneumonic pasteurellosis and effective treatment. Animal owners should be aware of the risk of pneumonia in their herds through extension and should avoid predisposing factors which lead to pneumonia.

Impact

The findings of this study indicate that more than one bacterial agent associated with pneumonia in sheep. The study also indicates that Gram – positive bacteria play a role in pneumonic conditions in sheep which may need further investigation.

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References

El Dirani O.H., Abdul Jabar M., and Babikir B.I., (2009). Constrains in The Market Chains for Export of Sudanese Sheep and Sheep Meat to Middle East. Research report 16.

Department of agricultural Economics, University of Khartoum, the Sudan and *ILRI (International Livestock Research Institute)*, Nairobi, Kenya. pp: 1-11.

Casamitijana P. (1994). *Pathologie Pulmonarie Ovine. Bulletin* des GTV, 3 : 126-136.

Cathryne A. Goodwig-Ray., (2006). Pneumonia and pleurisy in sheep: Studies of prevalence, risk factors, vaccine efficacy and economic impact. *Ph. D. thesis. University of Massey*, Palmerstone North, New Zeeland, pp: 1-3.

Jones T. C. Hunt R. D. and King N. W. (1997). Veterinary Pathology. 6th. ed. *Willey-Blackwell Publication*, UK. :1392.

Lehmkuhl H.D., Brigg R.E., Sacco, R. E., Tatum F.M, (2007). Viruses as predisposing factors to bacterial pneumonia, respiratory diseases in Mountain sheep: *knowledge Gaps and future Research:* 154

Quinn P. J., Markey M. E., Carter W. J. Donnelly W. J. C. and Leonard F. C. (2001) Veterinary Microbiology and Microbial Diseases. *Blackwell Science LTD*, London : 137.

Shigidi M.T.A. (1976). The occurrence of Pasteurella haemolytica in the nasopharynx of healthy sheep in Sudan. *The Sudan Journal of Veterinary Science and Animal Production* 17(1) : 9

El-Sanousi S. M., Gameel A. A., Al- Nawawi F., Al-Shazly M. O. (1991). Association of Klebsiella organisms with pulmonary lesions in sheep. Revue. Elev. *Med. Vet. Pay strop*. 44(2): 161-164.

Biberstein E.L., Hirsh D. C., and Zee Y.C. (1999). Pasteurella In :*Veterinary Microbiology, Eds.. Blackwell Science Inc.* London :135.

Rhaymah M. S., Shamon J. N. and Al-Jawally E. A. (2001). Bacteriological and pathological study of pneumonia in fattening lambs Iraqi *Journal of Veterinary Science.* 14 (1): 29

Alton C. S. Ward., Glen C. Weiser., Bruce C. Anderson., Patrick J. Cummings., Karen F. Arnold. and Lynette B. Carbeil. (2006). Haemophilus somnus (Histophilus somni) in big-horn sheep. *Canadian Journal of Veterinary Research* 70 (1) : 34.

Yimer N. and Asseged B. (2007). Aerobic bacterial flora of the respiratory tract of healthy sheep slaughtered in Dessie Municipal abattoir, Northern Ethiopia REVUE D ELEVAGE MEDECINE VETERINAIRE (10) : 473.

El-Sanousi S. M. and Tag El-din M. H., (1971). Isolation of an organism resembling Actinobacillus actinoides from a pneumonic lung of sheep *The Sudan Journal of Veterinary Science And Animal Husbandry* 12(1) : 9.-0

Quinn P. J., Carter M. E., Markey B and Carter G. R., (1994). *Clinical Veterinary Microbiology*, WOLFE Publishing, UK. (14)

Barrow G. I., and Feltham R. K. A.(1993). Cowan & Steel´s Manual for The Identification of Medical Bacteria. Third ed.. Reprint (2003). *Cambridge University Press*, UK.(15)

Smith J. E. (1985). Laboratory Manual, Department of Microbiology, *University of Surrey*, UK.(16)

El-Sanousi S.M., Amna B., El-Azhari G. (1978). Association of Pasteurella multocida and Actionbacillus lignieressi with bronchopneumonia in sheep *The Sudan Journal of Veterinary Science And Animal Husbandry* 19 (2): 102.

Manal I. A. M. (1998). Study of bacterial flora of the upper respiratory tract of pneumonic sheep. *M.Sc. Thesis, U. of K. Sudan.*

Fadia M. K. (1998). Bacteria isolated from pneumonic lungs of sheep. *M. Sc. Thesis. U. of K. Sudan.*

Tabo M., Woldemeskel M. and Glopilo A.,(2001). outbreak of respiratory disease complex in sheep in central Ethiopia. *Tropical Animal Health and Production* 33 (5) : 355.

Zaitoun A. M. (2001). Clinical studies of pneumonic mycoplasmosis and pasteurellosis (concurrent infection) in a commercial sheep flock. *Assiut Veterinary Medicine Journal* 45 : 162.

Ozbey G. and Muz A. (2004). Isolation of aerobic bacterial agents from the lungs of sheep and goats with pneumonia and detection of Pasteurella multocida and Mannheimia haemolytica by Polymerase Chain Reaction Turkish Journal of

Veterinary Animal Science. 28 : 209.

Veterinary Education and Information Network (VEIN). (2008). Diseases of the respiratory system. Sheep Health and Production. www.acronymfinder.com/VEIN.html

Sayed A. M. (1997). Some bacteriological and mycological studies on sheep pneumonia at Assiut Governorate. *Assiut Vet. Med. J.* 36 : 36.

Ertan ORG. (2006). The pathologic and bacteriologic comparison of pneumonia in lambs *Turkish Journal of Veterinary Animal Science* 30: 593.

Porter J. F., Connor K. and Donachie W. (1994). Isolation and characterization of Bordetella – like bacteria from ovine lungs. *Microbiology*, 140 : 1672.

Al-Sultan I. I. (1995).). Bacterial isolation from pneumonic lungs in sheep. *Iraqi Journal of Veterinary Science* 8: 251-254.

Cynthia M. Kahn. and Scott L., (2005): Pasteurella and Mannheimia pneumonia In: The Merck Veterinary Manual 9th ed. Publisher: *Merck, Sharp and Dome, USA.*

BACTERIOLOGIC AND HISTOPATHOLOGIC STUDIES IN PNEUMONIC LAMBS IN SOKOTO, NORTH WESTERN NIGERIA

Ahmed A[1], Egwu G O[2], Garba H S[3], Magaji A A[4] and Tambuwal F M[5]
[1]National Veterinary Research Institute, Zonal Research Laboratory, Birnin Kebbi.
[2]Department of Veterinary Medicine, University of Maiduguri.
[3]Department of Veterinary Medicine, Surgery and Theriogenology;
[4]Department of Veterinary Public Health and Animal Production;
[5]Department of Veterinary Pathology and Microbiology, Usmanu Danfodiyo University, Sokoto, Nigeria.

Abstract

Mortality in lambs is known to be caused by pneumonia and other bacterial agents. Lamb pneumonia is an infectious disease of young lambs caused primarily by the bacterial organism Pasterella haemolytica. Lung samples recovered from dead lambs were tested bacteriologically and histopathologically to determine the associated bacterial agents as well as the type of pneumonia involved. Bacteria recovered from the samples included Pasteurella multocida, Staphyllococcus aureus, Arcanobacterium pyogenes, Closridium perfringes, Streptococcus, and Klebsiella pneumonia. Histopathologically, three types of pneumonia were recognized namely: Interstitial (46.51%), bronchopneumonia (32.56%) and fibrinous pneumonia (20.93%). It was observed that pneumonia is a major cause of mortality in lambs in Sokoto and pathogenic bacteria, particularly P. multocida are the most important bacterial agents in the pathogenesis of lamb pneumonia. It was also concluded that pneumonia affecting lambs can vary depending on agents of infection, environment, management, immune status of the lamb as well as treatment regime.

Key words: Pneumonic, Bacteriologic, Histopathologic, Lambs, Sokoto, Nigeria.

ETUDES BACTERIOLOGIQUES ET HISTOPATHOLOGIQUES CHEZ DES AGNEAUX PNEUMONIQUES A SOKOTO DANS LE NORD-OUEST DU NIGERIA

Resumé

La mortalité des agneaux est connue pour être causée par la pneumonie et d'autres agents bactériens. La pneumonie de l'agneau est une maladie infectieuse qui affecte de jeunes agneaux, causée principalement par la bactérie Pasterella haemolytica. Des échantillons de tissus pulmonaires prélevés sur des agneaux morts ont été soumis à des tests bactériologiques et histopathologiques afin de déterminer les agents bactériens responsables, ainsi que le type de pneumonie. Les bactéries identifiées dans ces échantillons comprenaient :Pasteurella multocida, Staphyllococcus aureus, Arcanobacterium pyogenes, Closridium perfringes, Streptococcus, et Klebsiella pneumonia. Le test histopathologique a révélé trois types de pneumonie, à savoir: la pneumonie interstitielle(46,51%), la bronchopneumonie (32,56%) et la pneumonie fibrineuse(20,93%). On a constaté que la pneumonie est une cause majeure de mortalité chez les agneaux à Sokoto, et que les bactéries pathogènes - en particulier P. multocida - sont les agents les plus importants dans la pathogenèse de la pneumonie de l'agneau. On a également conclu que les types de pneumonie affectant les agneaux peuvent varier en fonction de l'agent étiologique, de l'environnement, de la prise en charge, du statut immunitaire de l'agneau ainsi que du régime de traitement.

Mots-clés: Pneumonique, Bactériologique, Histopathologique, Agneaux, Sokoto, Nigeria.

Corresponding Author: abdahmed65@gmail.com.

Introduction

Lamb pneumonia is an infectious disease of young lambs caused primarily by the bacterial organism Pasterella haemolytica (Hartwig, 2000) and is characterized by fever, increased respiratory rate, failure to nurse and death in untreated cases. The disease may occur in all ages of sheep (Hartwig, 2000) but is especially common in newborn and feedlot lambs (Schoenian, 2009; Veterinary Education and Information Network (VEIN), 2009) where it causes the most economic loss. It is a major cause of lamb mortality in many sheep flocks and can be a particular problem in artificially reared lambs in dairy flocks (Thoney *et al.,* 2002). This respiratory syndrome is a complex disease involving the interaction between the environment, microorganisms and the host (Oruc, 2006; Lacaste *et al.,* 2008). Lesions of pneumonia are seen frequently at necropsy in sheep of all ages.

Several bacterial organisms such as Pasteurella haemolytica, P. multocida, Staphylococcus, Mycoplasma spp, Actinobacillus lignieresii, Corynebacterium equi, Klebsiella spp, Escherichia coli, Neisseria spp and Actinomyces pyogenes have all been recorded from the lungs of sheep with pneumonia (Harizoglu, 1997; Oruc, 2006; Goodwin, 2009; VEIN, 2009). These bacteria are common in sheep flocks, and the problem strains usually can be found in the nasal passages and tonsils of adult and often healthy appearing ewes (Shulaw, 2009). These bacteria are secondary invaders but are important in increasing the severity of the clinical and pathological signs of pneumonia. Transmission to the lamb is usually by aerosolized droplets, containing the bacteria.

The spectrum of histological form of pneumonic diseases vary from mild to severe, acute to chronic and proliferative interstitial to being exudative (Dungworth, 1985; Daniel *et al.,* 2009; VEIN, 2009). Grossly, in acute bronchopneumonia which is associated with bacterial infections (Pasteurella spp), the condition is severe, acute and exudative form. The lungs are swollen and consolidated appearing with bright purple-red patches which are solid. Some areas may contain greenish brown areas of necrosis. When excised, the lungs exude a frothy hemorrhagic fluid. In less severe cases the cut cranial lobes are grayish-pink, raised and consolidated. The cut surface is dense with thickened septa (Oruc, 2006, VEIN, 2009). Interstitial pneumonia tends to be infectious in nature with viruses, fungi, bacteria and parasites being the causes. Mycoplasma spp are particularly of importance in the pathogenesis of the condition (Blood and Radostits, 1987; VEIN, 2009). Histologically, there is enlargement and proliferation of the alveolar epithelium, alveolar edema and interstitial tissues. There is aggregation of lymphocytes around the alveoli, blood vessels and bronchioles (Blood and Radostits, 1987). However, there is absence of acute inflammatory reaction or tissue necrosis. Varying degree of damage to the lung is caused depending on the nature and intensity of host parasite interaction. This paper reports on bacteria associated with pneumonia in lambs and the nature of pneumonia in the affected lungs.

Materials and Methods

The study was conducted in Sokoto, the capital of Sokoto state, located in the north western part of Nigeria over one year period (November, 2006 - October, 2007). Sheep flocks, totaling ten in number were randomly selected. Total sheep population in the various selected flocks was 624 out of which 246 were lambs. Animals were kept under semi intensive management system with varying form of supplementation. Visits were made to the flocks every fortnight to collect lambs that died during the period. On occasions, the flock owners contacted the first author through telephone to give information of lamb death in the flocks. A total of eighty two lambs that died or were born dead during the study period were picked and transported in icebox to the postmortem room of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto for postmortem. Postmortem was carried out routinely according to standard procedures described by (Taiwo, 2005).

Bacterial Culture and Identification: Samples from pneumonic lungs were directly placed into peptone water and incubated overnight at 37°C as pre enrichment. A loop-

ful from each of the peptone water was then cultured onto MacConkey agar (MCA) (Oxoid, UK) and 5% Sheep Blood Agar (SBA) plates. All the cultured plates were incubated aerobically and anaerobically at 37°C for 24-48 hours. Suspect colonies were carefully picked and sub cultured on blood agar and nutrient agar and incubated for 24h at 37°C to obtain pure bacterial cultures. A stock culture from each isolate was then stored at 4°C and later identified based on morphological appearance and Grams stain. Confirmation of isolates was done using standard biochemical procedures (Cowan and Steel, 1993; Quinn *et al.,* 2003).

Histopathological studies/examinations: After gross examination, lung tissue specimens were fixed in 10% buffered formalin solution. Following routine histopathological processing of the specimens, 5µ paraffin sections were then stained using hematoxilin-eosin stains (H-E). This was as described by (William, 1993). Transverse sections from each lung were later examined microscopically to determine the presence and severity of pneumonia.

Results

Out of 41 lung tissues bacteriologically tested, a total of 191 bacterial organisms were isolated from the affected lungs. These included Pasteurella multocida which was the most frequently isolated bacterial pathogen 61 (31.94%). This was followed by Staphylococcus aureus 39 (20.42%). Other bacteria isolated and their prevalence included Escherichia coli 26 (13.61%), Arcanobacterium pyogenes 22 (11.52), Clostridium perfringes 19 (9.95%), Streptococcus species 15 (7.85%) and Klebsiella pneumonia 9 (4.71%) Table1.

Histopathologically, three types of pneumonia were identified from the 43 lung tissues analyzed. In total, 20 cases (46.51%) were evaluated as interstitial pneumonia which was the most predominant type. Microscopically, marked vascular congestion with expansion of the alveolar spaces was observed. There was marked infiltration of the interstitium by chronic inflammatory cells predominantly lymphocytes (Plate 1). The second type pneumonia observed was bronchopneumonia seen in 14 cases (32.56%). In histological examination, mild to moderate congestion of the parenchymal blood vessels was observed. The alveolar spaces appear expanded. The alveolar lamina was with filled fibrinous exudates and neutrophilic infiltration forming aggregates (Plate 2). Fibrinous pneumonia was the third type of pneumonia and the least in terms of occurrence, observed only in 9 cases (20.93%). Histopathologically, there were hyperemic, fibrinous as well as neutrophilic areas that were seen together. The inter- alveolar spaces were enlarged. The lymphatic capillaries were obstructed with fibrin thrombosis (Plate 3).

Discussion

Following bacteriological analyses of the pneumonic lung tissues in this study, bacteria were isolated from 41 (52.56%) of these lung specimens. Several authors (Harizoglu et al. 1997; Sharif et al. 2005; Daniel et al. 2009) have reported the presence of bacterial pathogens in the lungs of dead lambs. This data indicates that bacterial agents are among the most important causes of pneumonia in lambs. In the present study, Pasteurella multocida was the most predominant pathogen (31.94%) and this is similar to the findings of (Harizoglu et al. 1997) and (Goodwin et al. 2005) who reported 30.42% and 34.73% respectively. Higher values have however been reported by (Sharif et al. 2005) and (Oruc, 2006). The variation may be attributed to difference in environment of these studies. Alternatively it may be the result of the use of antibiotics in flocks sampled in this study since animals are kept under semi intensive system.

Other bacterial organisms isolated in this study were S. aureus, E. coli, Arcanobacterium pyogenes, Cl. perfringes, Sreptococcus spp and Klebsiella pneumonia. This agrees with the findings of (Otesile et al. 1982) and (Goodwin, 2009) who also reported isolating these organisms in sheep. S. aureus is of public health significance since it is involved in mastitis worldwide (Quinn et al. 1994). Food poisoning can result from S. aureus infections with affected person showing signs of nausea, abdominal cramps and diarrhoea. The isolation of E. coli from the affected lungs

Table 1: Bacteria isolated from the pneumonic lungs of lambs

Bacteria	Number Isolated	Percent of Total
Staphylococcus aureus	39	20.42
Streptococcus spp	15	7.85
Escherichia coli	26	13.61
Pasteurella multocida	61	31.94
Klebsiella pneumoniae	9	4.71
Clostridium perfringes	19	9.95
Arcanobacterium pyogenes	22	11.52
Total	**191**	**100.0**

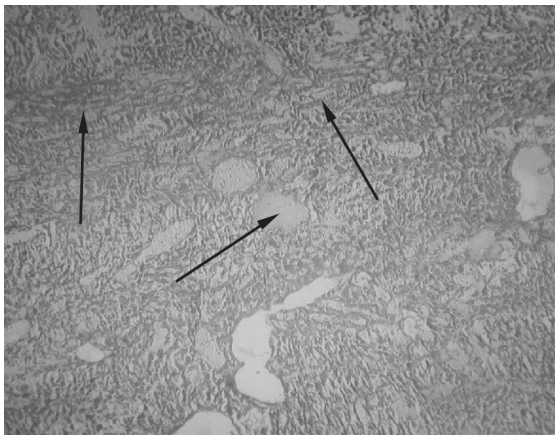

Plate 1: The lung of a four day old balami lamb showing interstitial pneumonia. Marked vascular congestion with expansion of alveolar spaces and lymphocytes infiltration.

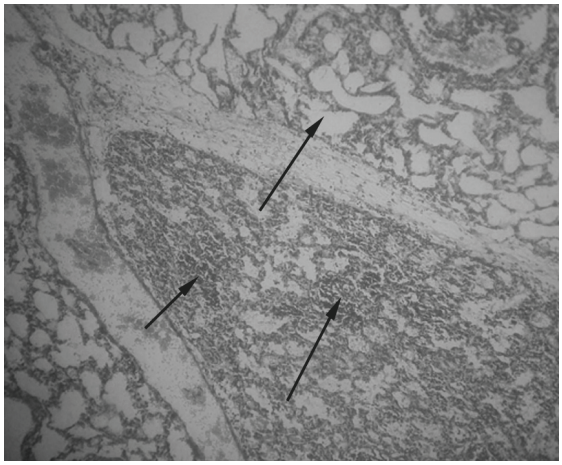

Plate 2: Lung of a seven day old Uda lamb showing bronchopneumonia. Expanded alveolar spaces with fibrinous exudates and neutrophilic infiltration (arrowed).

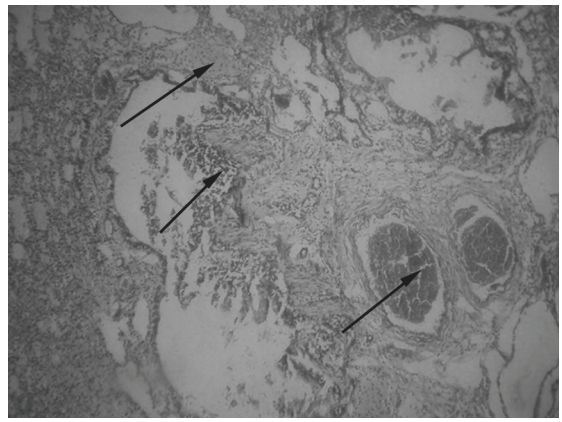

Plate 3: Lung of a one month old Uda lamb showing fibrinous pneumonia. Fibrinous content, peribronchial cellular infiltration congested blood vessels (arrowed).

was interest because of its being a member of enterobacteriaceae group. However, according to (Quinn *et al.,* 1994) it is an opportunistic organism and is frequently responsible for most clinical mastitis in domestic animals. Infection of the young lamb may occur during suckling.

In the present study, interstitial pneumonia was the most frequently observed type of pneumonia. This was followed by bronchopneumonia and fibrinous pneumonia. These types of pneumonias are slightly different from those reported by (Oruc, 2006) in Turkey, where fibrinous pneumonia was the frequently encountered type whilst (Bekele *et al.,* 1992) and (Saglam *et al.,* 1997) reported verminous pneumonia as being the most prevalent in their studies. The variations may be due to geographical influence suggestive of the possibility that pneumonia types can vary from region to region depending on climatic influences (Oruc, 2006; Lacasta *et al.*

2008). However, the histological appearances observed in the various pneumonia types in the present study were generally similar to those reported in previous studies (Goodwin *et al.,* 2005; Oruc, 2006; VEIN, 2009).

Although the recovery of mycoplasma was not carried out, its role cannot be determined in the pathogenesis of pneumonia in this study. However, the preponderance of interstitial pneumonia in the present study may suggest the involvement of mycoplasma organisms. This pathogen is reported to be frequently associated with this pneumonia (VEIN, 2009). Mycoplasma pneumonia is usually mild with mild clinical signs but is exacerbated by other bacteria infecting the lungs and cause more severe response and signs (Sullivan *et al.,* 1973a,b,). The isolation of pathogenic Pasteurella multocida from the affected lungs underscores its significance as the major organism associated with pneumonias in lambs (Harizoglu *et al.,* 1997). On the other hand, we note that the isolations of other opportunistic bacteria in the like E. coli and S. aureus may indicate the sign of secondary bacterial complications in the in the development of the pneumonic conditions observed. According to (Dungworth, 1985) and (Oros *et al.,* 1997), some viral agents are known to play a major role in the epidemiology and pathogenesis of lamb pneumonia especially with regards to the onset of the infection. In the present study however, the role of viruses have not been determined.

Conclusions

It was observed that pneumonia is a major cause of mortality in lambs in Sokoto and pathogenic bacteria particularly P. multocida are the most important bacterial agents in the pathogenesis of lamb pneumonia. It was also observed that pneumonia affecting lambs can vary depending on agents of infection, environment, management, immune status of the lamb as well as treatment regime.

Impact of the Study

Lamb pneumonia is an infectious disease of young lambs which is caused by bacterial organisms and is characterized by fever, increased respiratory rate, and failure to nurse as well as death. Cold, wet and windy environments are particularly problematic to the young lambs and may increase the susceptibility of the young animals to the disease. The ability of farmers to recognize the early signs of the condition especially where there is associated mortalities in the young lambs can help in the prevention of the disease thus, reducing the ensuring death from otherwise affected animals. The need for farmers to ensure that they provide a clean, dry and warm environment for the young animals is also of importance to prevent infection by the causal agents of pneumonia. And not just the environment, ensuring that the young newborn lambs suckle from dams with clean udders is also of importance since infection of the young lambs can also occur during suckling.

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References

Schoenian S, 2009. SHEEP 201. Care of newborn lambs. In: A beginner's guide to raising sheep. Visited August, 18th 2009 from http://www.sheep101.info/201/newborns.htm 1

Bekele T, Kasali O B and Woldeab T, 1992. Causes of lamb mortality in the Ethiopian highlands. *Veterinary Research Communication*, 16: 415 – 424.

Blood D C and Radodtits O M, 1987. A Textbook of the diseases of cattle, sheep, pigs, goats and horses. 7th ed. ELBS, *Bailliere and Tindall,* UK: pp 1293.

Cowan S T and Steel J K, 1993. Manual for the identification of medical bacteria. 3rd ed. (Brown, G. I. and Feltham, R. K. A. eds). *Cambridge University. Press*, pp: 226.

Daniel J A, Held J E, Brake D G, Wulf D M and Epperson W B, 2006. Evaluation of the prevalence and onset of lung lesions and their impact on growth of lambs. *American Journal of Veterinary Research*. 67:890 - 894.

Dungworth D L, 1985. The Respiratory System. In: Jubb K V F, Kennedy P C, Palmer N, Eds. Pathology of Domestic Animals. 3rd ed., Vol. 2, Academic Press, New York, pp: 451-532.

Goodwin K A, 2009. Pneumonia and pleurisy in lambs. Massey University and AgResearch, Agriculture NZ, Epicentre. Visited July, 23rd, 2009 from http://www.ceresfarm.co.nz/pneumonia.htm

Goodwin K A, Jackson R, Brown C, Davies P R, Morris R S and Perkins N R, 2005. Heat stress as a manageable risk factor to mitigate pneumonia in lambs. *New Zealand Veterinary Journal*, 53: 91- 92.

Harizoglu R, Diker K S, Gulbahar M Y, Akan M and Guvenc T, 1997. Studies of the pathology and microbiology of pneumonic lungs of lambs. Dtsch. Tierarztl. Wochenschr, 101: 441-443

Hartwig N R, 2000. Sheep health: Fact sheet No. 10. Iowa State University, University Extension Service.

Lacasta D, Ferrer L M, Ramos J J, Gonzales J M and De les Heras M, 2008. Influence of climatic factors on the development of pneumonia in lambs. *Small Ruminant Research*, 80 (1-3): 28-32.

Oros J, Fernandez A, Rodriguez J L, Rodriguez F and Poveda JB, 1997. Bacteria associated with enzootic pneumonia in goats. *Journal of Vet Med B*. 44:99.

Oruc E, 2006. The pathologic and bacteriologic comparison of pneumonia in lambs. *Turkish Journal of Veterinary and Animal Science*, 30:593-599.

Otesile E B, Kasali O B and Babalola M L, 1982. Mortality in sheep on the University of Ibadan teaching and research farm, Ibadan, *Nigeria. Bulletin of Animal Health Production in Africa*, 30:235.

Quinn B G, Carter M E and Markey B K, 2003. Concise Review of Veterinary Microbiology. Blackwell Publ. Ltd. Oxford, pp: 153.

Saglam Y S, Bozoglu H, Bas A T, 1997. Erzurum ve cevresinde kuzu enzootic pnomonileri uzerinde bakteriyolojik ve patolojik incelemeler. Etlik Vet. Mikrobiyol, 10:1-17

Sullivan N D, St George T D and Horsfall N, 1973a. A proliferative interstitial pneumonia of sheep associated with mycoplasma infection: 1. Natural history of the disease in a flock. *Australian Veterinary Journal*, 49 (57): 57-62.

Sullivan N D, St George T D and Horsfall N, 1973b. A proliferative interstitial pneumonia of sheep associated with mycoplasma infection: 2. The experimental exposure of young lambs to infection. *Australian Veterinary Journal*, 49 (63): 63-63.

Sharif L, Obeidat J and Al-Ani F, 2005. Risk factors for lamb and kid mortality in sheep and goat farms in Jordan. *Bulgarian Journal of Veterinary Medicine*, 8(2): 99.

Shulaw B, 2009. Management considerations to lower lamb mortalities. The Ohio State University: Sheep Team. Visited August, 17th, 2009 from http://sheep.osu.edu/2009/01/13/management considerations to lower lamb mortalities.

Taiwo V O, 2005. A manual for necropsy procedures for veterinary medical students and clinicians. Dabfol Print & Pack Limited, Ibadan, Nigeria, pp: 123.

Thonney M L, Smith M C and Mateescu R G, 2002. Vaccinations of ewes and lambs against para-influenza3 to prevent lamb pneumonia. In the Proceedings of the 8th Great Lakes Dairy Sheep Symposium, 7- 9 November, Cornell University: pp: 88-94.

VEIN, 2009. Sheep health and Production: Diseases of the respiratory system. Veterinary Education and Information Network. *University of Sydney, Faculty of Veterinary Science*.

William J B, 1993. Paraffin technique. In: Applied Veterinary Histology. (3rd ed). Mosby Yearbook, pp: 5-7.

PREVALENCE OF HELMINTHIC INFECTIONS AMONG WILD ANIMALS IN YANKARI GAME RESERVE, NIGERIA

Mbaya A W[1]*, Chuchan G K[1], Ballah F[1] and Garba B[1]
[1]Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B. 1069 Maiduguri, Nigeria

Abstract

The prevalence of helminthic parasites among wild animals in Yankari Game Reserve, Nigeria was assessed for the first time. Out of a total of 397 wild animals belonging to 3 groups and 17 species examined, the infection was significantly ($P < 0.05$) high among small spotted genet cats (Genetta genetta) 3(33.33%), baboons (Papio anubis) 51(47.66%), red pattas monkeys (Erythrocebus pattas) 3(33.33%), tantalus monkeys (Cercopethicus aethiopes tantalus) 5(100%), waterbucks (Kobus deffasa) 10(96.92%), buffalos (Cyncerus caffer) 6(60%) and Hippopotami (Hippopotami amphibious) 3(100%). This is in comparison to lower prevalence rates encountered among the lions (Panthera leo) 12(27.91%), African civet cats (Viverra civetta) 5(15.15%), serval cats (Felis serval) (14.29%), Temminek's golden cats (Felis temminekii) 1(25%) and crocodiles (Crocodylus niloticus) 3(7.89%). Similarly, it was lower among the Proboscidae / Artiodactyla, African elephants (Loxodonta africana) 11(16.42%), bushbucks (Tragelaphus scriptus) 1(18.0%), warthogs (Phacochoerus aethiopes) 5(26.32%) and hartebeests (Alcelaphus buselaphus) 2(22.22%). Among the primates and Artiodactyla/ Proboscidae, the males were more infected than their female counterparts ($p<0.05$). However, among the carnivores/reptiles the females were significantly ($p<0.05$) more infected than their male counterparts. According to age, the young animals were more infected than their adult counterparts ($p<0.05$). Mean faecal egg counts revealed that the intensity of infection was generally low to moderate in most animal groups. However, among the lions (Panthera leo) egg counts of 225.0 ± 0.41 due to Strongyloides and 237.5 ± 0.42 due to Strongyle infections were significantly high ($P<0.05$). Similarly, the baboons (Papio anubis) and African elephants (Loxodonta africana) had 203.3 ± 0.01 due to Ascaris and 450 ± 21.21 due to Strongyloides respectively. The warthogs (Phacochoerus aethiopes) and hippopotami (Hippopotami amphibious), counts of 220 ± 1.85 and 200 ± 14.14 due to Toxoascaris and Dicrocoelum species were encountered respectively. Faecal culture and larval recovery revealed that Strongyloides canis and Ancylostoma caninum larvae were common among the carnivores. For the primates, Stronyloides stercoralis and Ancylostoma duodenale were encountered while, Haemonchus contortus, Stronyloides pappilosus, Trichostrongylus colubrioformis and Oesophagostamum columbianum were encountered among the Artiodactyla/ Proboscidae. In conclusion the wild animals harboured medical and veterinary parasites of various intensities.

Key words: prevalence, intensity, helminthic infections, wild animals, Yankari Game Reserve, Nigeria

PREVALENCE DES INFECTIONS HELMINTHIQUES CHEZ LES ANIMAUX SAUVAGES EN YANKARI GAME RESERVE, NIGERIA

Résumé

La prévalence des parasites des helminthes chez les animaux sauvages dans la reserve de Yankari Game Reserve, le Nigeria a été etudiee pour la première fois. Sur un total de 397 animaux sauvages appartenant à 3 groupes et 17 espèces examinées, l'infection a été significativement ($P <0,05$) élevé chez les petits chats genette tachetée (Genetta genetta) 3 (33,33%), les babouins (Papio anubis) 51 (47,66 %), le rouge des singes pattas (Erythrocebus pattas) 3 (33,33%), des singes Tantalus (Cercopethicus aethiopes tantalus) 5 (100%), cobes (Kobus deffasa) 10 (96,92%), des buffles (Cyncerus caffer) 6 (60%) et les hippopotames (amphibies hippopotames) 3 (100%). C'est en comparaison avec les taux de prévalence plus faible rencontrée chez les lions (Panthera leo) 12 (27,91%), Afrique civette (Viverra civetta) 5 (15,15%), les chats serval (Felis serval) (14,29%), des chats dorés Temminek de (Felis temminekii) 1 (25%) et les crocodiles

*Correspondence Author: awmbaya@yahoo.com.

(Crocodylus niloticus) 3 (7,89%). De même, il a été plus faible chez les proboscidiens / Artiodactyles, les éléphants d'Afrique (Loxodonta africana) 11 (16,42%), phacochères (Tragelaphus scriptus) 1 (18,0%), les phacochères (Phacochoerus aethiopes) 5 (26,32%) et bubales (Alcelaphus buselaphus) 2 (22,22%). Parmi les primates et les artiodactyles / proboscidiens, les mâles étaient plus infectées que leurs homologues féminins ($p < 0,05$). Cependant, parmi les carnivores / reptiles les femelles étaient significativement ($p < 0,05$) plus infectées que leurs homologues masculins. Selon l'âge, les jeunes animaux étaient plus infectées que leurs homologues adultes ($p < 0,05$). Mean nombre d'œufs fécaux a révélé que l'intensité de l'infection a été généralement faible à modéré dans la plupart des groupes d'animaux. Cependant, parmi nombre d'œufs que les lions (Panthera leo) de 225,0 ± 0,41 due à Strongyloides et 237,5 ± 0,42 dus à des infections Strongyle étaient significativement élevé ($P < 0,05$). De même, les babouins (Papio anubis) et les éléphants d'Afrique (Loxodonta africana) avait 203,3 ± 0,01 due à Ascaris et 450 ± 21,21 en raison de Strongyloides respectivement. Les phacochères (Phacochoerus aethiopes) et les hippopotames (amphibies hippopotames), compte de 220 ± 200 ± 1,85 et 14,14 en raison des espèces et des Toxoascaris Dicrocoelum ont été rencontrées respectivement. Coproculture et la récupération des larves a révélé que Strongyloides canis et larves Ancylostoma caninum étaient courants chez les carnivores. Pour les primates, Stronyloides stercoralis et Ancylostoma duodenale ont été rencontrées pendant, Haemonchus, Stronyloides pappilosus, colubrioformis Trichostrongylus et Oesophagostamum columbianum ont été rencontrés chez les artiodactyles / proboscidiens. En conclusion, les animaux sauvages abritait les parasites médicaux et vétérinaires de différentes intensités.

Mots-clés: Prévalence, intensité, helminthiases, les animaux sauvages, Yankari Game Reserve, au Nigeria

Introduction

Free-living wild animals have been reported to harbour various types of helminth parasites (Soulsby, 1982). Under free-roam, in their natural habitat, they often live at equilibrium with the parasites without manifesting overt disease (Young, 1977; Mbaya, 2007). However, adverse conditions of stress such as draught and starvation often compromised their innate resistance leading to clinical helminthosis (Mbaya *et al.,* 2008).

With the increased global concern towards the in-situ conservation of wildlife, coprological survey of helminth parasites of wildlife have been investigated in the Serengeti and Ngorongoro crater in East Africa (Christine and Muller-Graf, 1995), Eastern Cape Province (Boomer *et al.,* 1991) and in Europe (Lynn and Sussane, 2004; Barbosa *et al.,* 2005; Meshram *et al.,* 2008). Similar surveys have been reported among captive wild animals in Nigeria (Enyinihi, 1972; Isoun *et al.,* 1972; Bamidele and Ogunrinade, 1980; Nwosu, 1995; Mbaya and Aliyu, 2006; Mbaya *et al.,* 2006), among free-living wildlife in Kainji Lake National Park, Central Nigeria (Ogunji *et al.,* 1982; Crockett, 1983) and in the arid region of north-eastern Nigeria (Mbaya *et al.,* 2006; Mbaya and Aliyu, 2007).

However, despite the number of research work on the parasites of wildlife in Nigeria, information is totally lacking regarding helminthic infections of wild animals in the Yankari Game Reserve, Nigeria the nation's first and largest wildlife conservation area. In this study, for the first time, the prevalence and intensity of helminthic infections among free-living wildlife in Yankari Game Reserve, Nigeria was investigated.

Materials and Methods

Study site

This study was conducted in Yankari Game Reserve covering an area of about 2,250.10 sq km. It is situated between latitude 9'N and longitude 10'E within Duguri Pali and Gwana Districts of Alkaleri Local Government Area of Bauchi state, Nigeria. The Reserve has 52 identified species of mammals including elephants, making it the single largest elephant concentration site in West Africa. Also present in the Park are 153 known species of birds, 147 of fish, and several reptiles.

Sample collection

The study was conducted between January 2009 and December 2011 involving a total of 397 free-living wild animals of 17 different species. Among the carnivores/

reptiles, 43 lions (Panthera leo), 33 African civet cats (Viverra civetta), 9 small spotted genet cats (Genetta genetta), 7 serval cats (Felis serval), 4 Temminek's golden cats (Felis temminekii) and 38 crocodiles (Crocodylus niloticus) were examined. Among the primates, 107 baboons (Papio anubis), 9 red pattas monkeys (Erythrocebus pattas), and 5 tantalus monkeys (Cercopethicus aethiopes tantalus) were examined. Similarly, among the Proboscidae/ Artidactyla, 67 African elephants (Loxodonta africana), 10 bushbucks (Tragelaphus scriptus), 13 waterbucks (Kobus deffasa), 10 buffalos (Cyncerus caffer), 19 warthogs (Phacochoerus aethiopes), 3 hippopotami (Hippopotami amphibious), 9 hartebeests (Alcelaphus buselaphus) and 1 oribi (Oribi oribi) were examined. Randomly selected freshly passed faecal samples opportunistically deposited were collected with the help of 20 game trackers. The samples were labelled demographically according to specie, sex and age. To determine the specie, sex and age based on faecal sampling, a high powered binoculars (Nikon) was used to view the animals from a short distance. A huge data base was then generated by computer to later identify the animals according to species, sex and age based on natural body markings, ear notches, scars or special defects. The animals were followed by the trackers in a Toyota 4x4 sport utility vehicle to collect faecal samples from the previously identified animals as soon as they are dropped and transported in 10% formalin to the Parasitology Laboratory of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria for coprological examination. The coordinates of each collection, was recorded using the Global Positioning System (GPS) hand-held receiver. The animals were not subjected to any form of capture or distress during the study. Animal welfare was strictly observed according to international guidelines.

Laboratory investigations

Each faecal sample was examined for helminth ova by the direct faecal smear, sedimentation and floatation methods while faecal egg counts per gram of faeces (epg) was used to determine the intensity of the infection by the modified McMaster technique using saturated sodium chloride solution as the floating medium (MAFF, 1977). Faecal culture and larval recovery were done using the modified Baerman's technique (Hanseen and Perry, 1994). The identification of helminth ova and infective larvae were done using standard parasitological criteria (Sloss and Kemp, 1978; Soulsby, 1982).

Statistical analysis

The X2 ICC test adjusted for intra-cluster correlation was used to judge differences in risks between various strata (Donald and Donner, 1988).

Results

The prevalence of helminthic infections among the wild animals examined in Yankari Game Reserve, Nigeria is presented in Tables 1, 4 and 7. A total of 397 free-living wild animals were examined of which, 134 of them were carnivores/reptiles out of which, 25(18.66%) of them were shedding the ova of at least one gastrointestinal parasite in their faeces. Similarly, out of the 121 primates examined, 59(48.76%) of them were infected. Among the 132 Proboscidae and Artiodactylae examined, 38(28.79%) of them were also infected. Between the groups, out of the 9 small spotted genet cats (Genetta genetta), 107 baboons (Papio anubis), 9 red pattas monkeys (Erythrocebus pattas), 5 tantalus monkeys (Cercopethicus aethiopes), 13 waterbucks (Kobus deffasa), 10 buffalos (Cyncerus caffer), and 3 hippopotami (Hippopotami amphibious) examined, 3(33.33%), 5(100%), 10(96.92%), 6(60%) and 3(100%) were respectively and significantly ($P<0.05$) more infected than 12(27.91%) of the 43 lions (Panthera leo), 5(15.15%) of the 33 African civet cats (Viverra civetta), 1(14.29)% of the 7 serval cats (Felis serval), 1(25%) of the 4 Temmineki's golden cats (Felis temminekii), 3(7.89%) of the 38 crocodiles (Crocodylus niloticus), 11(16.42%) of the 67 African elephants (Loxodonta africana), 1(18.0%) of the 10 bushbucks (Tragelaphus scriptus), 5(26.32%) of the 19 warthogs (Phacochoerus aethiopes), 2(22.22%) of the 9 hartebeests (Alcelaphus buselaphus) examined. Meanwhile, no parasite was encountered in the single oribi (Oribi oribi) examined.

Table 1: Prevalence of gastrointestinal parasitic infections among wild carnivores and reptiles in Yankari Game Reserve, Nigeria

Species of carnivores	No. Examined	No. Infected (%)
Lions (*Panthera leo*)	43	12(27.91)[a]
African civet cats (*Viverra civetta*)	33	5(15.15)[b]
Small spotted genet cats (*Genetta genetta*)	9	3(33.33)[a]
Serval cats (*Felis serval*)	7	1(14.29)[b]
Temminek's golden cats (*Felis temminekii*)	4	1(25)[a]
Crocodiles (*Crocodylus niloticus*)	38	3(7.89)[c]
All carnivores	134	25(18.66)

a, b, c superscripted values in third column differed significantly (P < 0.05)

Table 2: Prevalence of gastrointestinal parasitic infections among wild carnivores and reptiles in Yankari Game Reserve Nigeria, according to sex

Species of carnivores	No. Examined		No. Infected (%)	
	Male	Female	Male	Female
Lions (*Panthera leo*)	20	23	5(25)[a]	7(30.4)[b]
African civet cats (*Viverra civetta*)	15	18	2(13.33)[a1]	3(16.67)[b1]
Small spotted genet cats (*Genetta genetta*)	4	5	1(25)[a2]	2(40)[b2]
Serval cats (*Felis serval*)	4	3	1(25)[a3]	0(0)[b3]
Temminek's golden cats (*Felis temminekii*)	2	2	0(0)[a4]	1(50)[b4]
Crocodiles (*Crocodylus niloticus*)	15	23	1(6.67)[a5]	2(8.70)[b5]
All carnivores	60	74	10(16.67)	15(20.2)

Different superscripted values between rows in the 4th and 5th column differed significantly (P < 0.05)

Table 3: Prevalence of gastrointestinal parasitic infections among wild carnivores and reptiles in Yankari Game Reserve, Nigeria according to age

Species of carnivores	No. Examined		No. Infected (%)	
	Young	Adults	Young	Adults
Lions (*Panthera leo*)	21	22	4(19.05)[a]	8(36.36)[b]
African civet cats (*Viverra civetta*)	11	22	3(27.27)[a1]	2(9.09)[b1]
Small spotted genet cats (*Genetta genetta*)	2	7	1(50)[a2]	2(28.57)[b2]
Serval cats (*Felis serval*)	2	5	1(50)[a3]	0(0)[b3]
Temminek's golden cats (*Felis temminekii*)	1	3	1(100)[a4]	0(0)[b4]
Crocodiles (*Crocodylus niloticus*)	10	28	2(20)[a5]	1(3.57)[b5]
All carnivores	47	87	12(25.63)	13(14.94)

Different superscripted values between rows in the 4th and 5th column differed significantly (P < 0.05)

Table 4: Prevalence of gastrointestinal parasitic infections of primates in Yankari Game Reserve, Nigeria

Species of primates	No. Examined	No. Infected (%)
Baboons (Papio anubis)	107	51(47.66)[a]
Red pattas monkeys (Erythrocebus pattas)	9	3(33.33)[b]
Tantalus monkeys (Cercopethicus aethiopes tantalus)	5	5(100)[c]
All primates	121	59(48.76)

a, b, c superscripted values in 3rd column differed significantly (P < 0.05)

Table 5: Prevalence of gastrointestinal parasitic infections of primates in Yankari Game Reserve, Nigeria according to age

Species of primates	No. Examined		No. Infected (%)	
	Young	Adults	Young	Adults
Baboons (Papio anubis)	54	53	31(57.41)[a]	20(37.73)[b]
Red pattas monkeys (Erythrocebus pattas)	5	4	2(40)[a1]	1(25)[b1]
Tantalus monkeys (Cercopethicus aethiopes tantalus)	5	0	5(100)[a2]	0(0)[b2]
All primates	64	57	38(59.38)	21(36.84)

Different superscripted values between rows in the 4th and 5th column differed significantly (P < 0.05)

Table 6: Prevalence of gastrointestinal parasitic infections of primates in Yankari Game Reserve, Nigeria according to sex

Species of primates	No. Examined		No. Infected (%)	
	Male	Female	Male	Female
Baboons (Papio anubis)	50	57	30(60)[a]	21(36.84)[b]
Red pattas monkeys (Erythrocebus pattas)	3	6	2(66.66)[a1]	1(16.67)[b1]
Tantalus monkeys (Cercopethicus aethiopes tantalus)	0	5	0(0)[a2]	5(100)[b2]
All primates	53	68	32(60.38)	27(39.70)

Different superscripted values between rows in the 4th and 5th column differed significantly (P < 0.05)

The prevalence of infection among the wild carnivores/reptiles according to sex is shown in Table 2. The females 15(20.2%) were significantly ($p<0.05$) more infected than males 10(16.67%). Meanwhile among the primates and Artiodactyla/Proboacidae, 32(60.38%) and 22(31.43%) of the males were more infected ($p<0.05$) than 27(39.70%) and 16(25.81%) of their female counterparts respectively (Tables 6 and 9). Among the young carnivores/reptiles, primates and Artiodactyla/Proboscidae, the prevalence of 12(25.63%), 38(59.38%) and 25(41.67%) were significantly ($p<0.05$) more infected than 13(14.84%), 21(36.84%) and 13(18.09%) of their adult counterparts, respectively (Tables 3, 5, 8).

The various helminth parasites and their intensities among the carnivores/reptiles are presented in Table 10. The intensity of helminth infections depicted as total egg count (epg) among 12(27.91) of the 43 lions (Panthera leo) examined was 649.2 ± 0.09. Out of these, 2(16.67%) lions harboured Strongyloides with an intensity of 225.0 ± 0.41 and the helminth larvae recovered were Strongyloides canis (46.2 ± 2.40). Similarly, 6(50%) and 4(33.33%) of the lions harboured Toxoascaris and Strongyle with the intensities of 186.7 ± 0.37 and 237.5 ± 0.42 respectively. From the lions infected with Strongyle, the larvae of Ancylostoma caninum (144.2 ± 1.50) were recovered. Among the 33 African civet cats (Viverra civetta), 5(15.15%) of them were infected with an intensity of 250 ± 0.09. Out of these, 2(40%) had an intensity of 75.0 ± 108 due to Opisthorchis, 2(40%) with an intensity of 125.0 ± 1.40 due to Toxoascaris while 1(20%) had an intensity of 50 ± 0.88 due to Trichuris. No helminth larvae were recovered from this specie of wild cats. Among the 7 serval cats (Felis serval) and 4 Temminek's golden cats (Felis temminekii) examined, 1(14.29%) and 1(25%) were infected with intensities of 150 ± 12.25 and 50 ± 7.07 due to Trichuris and Toxoascaris respectively while, no helminth larvae were recovered. For the 38 crocodiles (Crocodylus niloticus) examined, 3(7.89%) of them harboured Fasciola with an intensity of 183.3 ± 4.79 with no larvae isolated.

The various helminth parasites and their intensities among the various primates examined are presented in Table 11. Out of the 107 baboons (Papio anubis) examined, 51(47.66%) were infected with a total intensity of 394.1 ± 0.01. Out of which, 26(50.98%), 10(19.61%) and 15(29.41%) were infected with 105.8 ± 0.01, 85.0 ± 0.41 and 203.3 ± 0.01 intensities due to Strongyloides, Hymenolepis and Ascaris respectively. Among those infected with Strongyloides, the larvae of Strongyloides stercoralis (160.6 ± 0.10) were recovered.

Table 7: Prevalence of gastrointestinal parasitic infections of wild Proboscidae and Artiodactyla in Yankari Game Reserve, Nigeria

Species of Proboscidae/ Artiodactyla	No. Examined	No. Infected (%)
African elephants (*Loxodonta africana*)	67	11(16.42)[a]
Bushbucks (*Tragelaphus scriptus*)	10	1(10.0)[a]
Waterbucks (*Kobus deffasa*)	13	10(96.92)[b]
Buffalos (*Cyncerus caffer*)	10	6(60)[c]
Warthogs (*Phacochoerus aethiopes*)	19	5(26.32)[d]
Hippopotami (*Hippopotami amphibious*)	3	3(100)[b]
Hartebeests (*Alcelaphus buselaphus*)	9	2(22.22)[e]
Oribis (*Oribi oribi*)	1	0(0)[f]
All Proboscidae and Artiodactyla	132	38(28.79)

a, b, c, d, e, f Superscripted values in 3rd column differed significantly (P < 0.05)

Table 8: Prevalence of gastrointestinal parasitic infections of wild Proboscidae and Artiodactyla in Yankari Game Reserve in Nigeria according to age

Species of Proboscidae/ Artiodactyla	No. Examined		No. Infected (%)	
	Young	Adults	Young	Adults
African elephants (*Loxodonta africana*)	30	37	6(20)[a]	5(13.5)[b]
Bushbucks (*Tragelaphus scriptus*)	4	6	1(25)[a1]	0(0)[b1]
Waterbucks (*Kobus deffasa*)	6	7	6(100)[a2]	4(57.14)[b2]
Buffalos (*Cyncerus caffer*)	5	5	4(80)[a3]	2(40)[b3]
Warthogs (*Phacochoerus aethiopes*)	8	11	4(50)[a4]	1(9.09)[b4]
Hippopotami (*Hippopotami amphibious*)	2	1	2(100)[a5]	1(100)[b5]
Hartebeests (*Alcelaphus buselaphus*)	4	5	2(50)[a6]	0(0)[b6]
Oribis (*Oribi oribi*)	1	0	0(0)[a7]	0(0)[b7]
All Proboscidae and Artiodactyla	60	72	25(41.67)	13(18.05)

Different superscripted values between rows in the 4th and 5th column differed significantly (P < 0.05)

Table 9: Prevalence of gastrointestinal parasitic infections of wild Proboscidae and Artiodactyla in Yankari Game Reserve Nigeria, according to sex

Species of Proboscidae/ Artiodactyla	No. Examined		No. Infected (%)	
	Male	Female	Male	Female
African elephants (*Loxodonta africana*)	33	34	7(21.21)[a]	4(12.12)[b]
Bushbucks (*Tragelaphus scriptus*)	5	5	1(20)[a]	0(0)[b]
Waterbucks (*Kobus deffasa*)	5	8	5(100)[a]	5(100)[b]
Buffalos (*Cyncerus caffer*)	7	3	3(42.86)[a]	3(100)[b]
Warthogs (*Phacochoerus aethiopes*)	10	9	3(30)[a]	2(22.22)[b]
Hippopotami (*Hippopotami amphibious*)	2	1	2(100)[a5]	1(100)[b5]
Hartebeests (*Alcelaphus buselaphus*)	7	2	1(14.29)[a]	1(50)[b]
Oribis (*Oribi oribi*)	1	0	0(0)[a]	0(0)[b]
All Proboscidae and Artiodactyla	70	62	22(31.43)	16(25.81)

Different superscripted values between rows in the 4th and 5th column differed significantly (P < 0.05)

Table 10: Various gastrointestinal parasites and their intensities among wild carnivores and reptiles in Yankari Game Reserve, Nigeria

Species of animals	Parasites encountered %	Helminth egg/gram ± S.D. (range)	Helminth larvae recovered ± S.D
Lions (*Panthera leo*) (n = 43)	(i) *Strongyloides* 2(16.67)[a]	225.0±0.41a (150-300)	*S. canis* (46.2 ± 2.40)
	(ii) *Toxoascaris* 6(50)[b]	186.7±0.37a (100-300)	
	(iii) *Strongyle* 4(33.33)[c]	237.5±0.42a (200-300)	*A. caninum* (144.2 ± 150)
Total	**12(27.91)**	**649.2 ± 0.09**	
African civet cats (*Viverra civetta*) (n = 33)	(i) *Opisthorcis* 2(40)[c]	75.0±1.08b (50-100)	Nil
	(ii)*Toxoascaris* 2(40)[c]	125.0 ±1.40a (100-150)	Nil
	(iii) *Trichuris* 1(20)a	50.0 ± 0.88b (50)	Nil
Total	**15(15.15)**	**250 ± 0.09**	
Small spotted genet cats (*Genetta genetta*) (n = 9)	(i) *Toxoascaris* 3(33.33)[d]	75.0 ± 3.06b (50-100)	Nil
Total	**3(33.3)**	**75.0 ± 3.06**	
Serval cats (*Felis serval*) (n= 7)	(i) *Trichuris* 1(14.29)[d]	150±12.25[a] (150)	Nil
Total	**1(14.29)**	**150 ± 12.25**	
Temminek's golden cats (*Felis temminekii*) (n = 4)	(i) *Toxoascaris* 1(25)[d]	50±7.07[b] (50)	Nil
Total	**1(25)**	**50 ± 7.07**	
Crocodiles (*Crocodylus niloticus*) (n = 38)	(i) *Fasciola* 3(7.89)[d]	183.3 ±4.79[a] (100-300)	Nil
Total	**3(7.89)**	**183.3 ± 4.79**	

Keys:

a, b, c, d superscripted values in column differed significantly ($P < 0.05$)

S. canis = Strongyloides canis

A. caninum = Ancylostoma caninum

Similarly, out of the 9 red pattas monkeys (Erythrocebus pattas) examined, 3(33.33%) of them were infected with an intensity of 66.67 ± 0.36 due to Strongyle which yielded the larvae of Ancylostoma duodenale (122.2 ± 0.49). Among the 5 tantalus monkeys (Cercopethicus aethiopes tantalus) examined, all 5(100%) were infected with an intensity of 110.0 ± 1.31, with the larvae of Ancylostoma duodenale (62.8 ± 0.99) recovered.

The helminth parasites and their intensities among the various Proboscidae and Artiodactyla examined are presented in Table 12. Out of the 67 African elephants (Loxodonta africana) examined, 11(16.42%) of them were infected with a total intensity of 755 ± 0.050. Out of which, 4(45.45%), 1(9.09%) and 6(54.55%) of them harboured Fasciola, Strongyloides and Strongyle with intensities of 130 ± 1.43, 430 ± 21.21 and 175 ± 1.18 respectively. The larvae of Strongyloides papillosus (100.2 ± 10.0) were recovered from those harbouring Strongyloides while Haemonchus contortus (152.6 ± 0.84) and Trichostrongylus colubriformis (78.0 ± 0.60) larvae were isolated from those harbouring Strongyle. Out of the 10 bushbucks (Tragelaphus scriptus) examined, 1(10%) harboured Dicrocoelium with an intensity of 50.0 ± 7.07 with no helminth larvae recovered. For the 13 waterbucks (Kobus deffasa) examined, 10(96.92%) were infected with a total intensity of 283 ± 0.36 out of which, 6(60%) and 4(40%) of them had intensities of 158 ± 1.13 and 125 ± 2.15 due to Fasciola and Dicrocoelium respectively with no helminth larvae recovered. Similarly, out of the 10 buffalos (Cyncerus caffer) examined, 6(60%) of them were infected with a total intensity of 200 ± 0.52. Out of which, 4(66.67%) and 2(33.33%) had 125 ± 2.15 and 75 ± 8.66

Table 11: Various gastrointestinal parasites and their intensities among wild primates and reptiles in Yankari Game Reserve, Nigeria

Species of animals	Parasites encountered %	Helminth egg/gram ± S.D. (range)	Helminth larvae recovered ± S.D
Baboons (*Papio anubis*) (n = 107)	(i) *Strongyloides* 26(50.98)[a]	105.8±0.01a (50-300)	*S. stercoralis* (160.6±0.10)
	(ii) *Hymenolepis* 10(19.61)[b]	85.0±0.41b (50-100)	Nil
	(iii) *Ascaris* 15(29.41)[b]	203.3±0.01a (50-500)	Nil
Total	**51(47.66)**	**394.1 ± 0.01**	
Red pattas monkeys (*Erythrocebus pattas*) (n = 9)	(i) *Strongyle* 3(100)[c]	66.67±0.36[b] (50-100)	*A. duodenale* (122.2±0.49)
Total	**3(33.33)**	**66.67 ± 0.36**	
Tantalus monkeys (*Cercopethicus aethiopes tantalus*) (n = 5)	(i) *Strongyle* 5(100)[c]	110.0 ± 1.31[a] (50-200)	*A. duodenale* (62.8 ± 0.99)
Total	**5(100)**	**110.0 ± 1.31**	

Keys:
a, b superscripted values in column differed significantly ($P < 0.05$). S. stercoralis = Strongyloides stercoralis
A. duodenale = Ancylostoma duodenale

intensities due to Fasciola and Dicrocoelium with no helminth larvae recovered. Out of the 19 warthogs (Phacochoerus aethiopes) examined, 5(26.32%) were infected with an intensity of 220 ± 1.85 due to Toxoascaris with no helminth larvae isolated. As for the 3 hippopotami (Hippopotami amphibious) examined, 2(66.67%) and 1(36.33%) had intensities of 100 ± 10.0 and 200 ± 14.14 due to Fasciola and Dicrocoelium respectively, with no helminth larvae recovered. For the 9 hartebeests (Alcelaphus buselaphus), 2(22.22%) were infected with a total intensity of 75 ± 8.66 due to Strongyle from which, the larvae of Haemonchus contortus (50 ± 2.5) and Oesophagostomum columbianum (160.2 ± 4.47) were recovered.

Discussion

The results of this study conducted for the first time in Yankari Game Reserve located in the Sudan savannah of north-eastern Nigeria revealed that the free-living wild animals in the area harboured a variety of gastrointestinal parasites. Some of the parasites encountered among the carnivores such as Toxoascaris and Ancylostoma species have been reported to cause severe outbreaks of toxoascariasis and ancylostosomosis among captive lions (Panthera leo) (Mbaya and Nwosu, 2004; Mbaya and Aliyu, 2006) or sometimes without causing overt signs among captive lions (Panthera leo) in the semi arid region of north-eastern Nigeria (Mbaya *et al.,* 2006). Some of the parasites recovered during the study have previously been reported in wild animals of similar species in other geographical zones of Nigeria (Enyinihi, 1972; Agbede and Yesufu, 1982; Crockett, 1983, Nwosu, 1995).

However, the occurrence of Toxoascaris in the Temminek's golden cats (Felis temminekii), Fasciola in the crocodiles (Crocodylus niloticus), hippopotami (Hippopotamus amphibious), African elephants (Loxodonta Africana), waterbucks (Kobus deffasa), buffalos (Cyncerus caffer) or Opisthorcis in the African civet cats (Viverra civetta) are being reported for the first time in such species living under free-living conditions in Nigeria.

The wild animals that were shedding Fasciola or Dicrocelium ova in their faeces were either aquatic or terrestrial species that wallow in mud around the river banks in the game reserve. It is therefore probable that they obtained the infection orally by ingesting encysted metacarcaria of the trematodes on grass blades. This was either through grazing as in the Proboscidae / Artiodactyla or ingested accidentally by the crocodiles (Crocodylus niloticus) while catching prey. The degree of infection among these species

might be associated with the heavy presence of the intermediate hosts (molluscs) of the genus (Lymnea) found along the river banks in the Game Reserve during the pilot study. The recovery of the larvae of Haemonchus contortus alongside Trichostrongylus papillosus or Oesophagostamum columbianum might be of serious concern due to the haematophagus activity of these species in causing anaemia in parasitic gastroenteritis complex (PGE) of small (Nwosu *et al.,* 2006) and large ruminants (Mbaya *et al.,* 2009) or red fronted gazelles (Gazella rufifrons) (Mbaya and Aliyu, 2007). On one hand, these high incidences of parasitic infections of veterinary importance among the wild animals might not be un-connected with the unwarranted incursion of nomadic herds into the game reserve in search of pasture. As such, the interaction of wild and domestic livestock in this high risk interface may pose problems to the exotic species in their natural habitat or vise versa. On the other hand, the occurrence of the parasites of medical importance among the primates might not be un-connected with indiscriminate defication by local communities living on fringes of the park. The occurrence of some of the parasites such as Toxoascaris, Ancylostoma, Trichuris,

Table 12: Various gastrointestinal parasites and their intensities among wild Proboscidae and Artiodactyla in Yankari Game Reserve, Nigeria

Species of animals	Parasites encountered %	Helminth egg/gram ± S.D. (range)	Helminth larvae recovered ± S.D
African elephants (*Loxodonta africana*) (n = 67)	(i) *Fasciola* 5(45.45)[a] (ii) *Strongyloides* 1(9.09)[a] (iii) *Strongyle* 6(54.55)[a]	130±1.43[a] (50-200) 450±21.21[d] (450) 175±1.18[c] (50-550)	Nil (i) *S. papillosus* (100.2±10.0) (i) *H. contortus* (152.6±0.84) (ii) *T. colubriformis* (78.0±0.60)
Total	11(16.42)	755 ± 0.050	
Bushbucks (*Tragelaphus scriptus*) (n=10)	(i) *Dicrocoelium* 1(100)[c]	50±7.07[b] (50)	Nil
Total	1(10.0)	50 ± 7.07	
Waterbucks (*Kobus deffasa*) (n = 13)	(i) *Fasciola* 6(60)[a] (ii) *Dicrocoelium* 4(40)[a]	158±1.13[a] (50-300) 125±2.15[a] (50-200)	Nil Nil
Total	10(96.92)	283 ± 0.36	
Buffalos(*Cyncerus caffer*) (n = 10)	(i) Fasciola 4(66.67)[a] (ii) Dicrocoelium 2(33.33)[b]	125±2.15[a] (50-200) 75±8.66[b] (50-100)	Nil Nil
Total	6(60)	200 ± 0.52	
Warthogs (*Phacochoerus aethiopes*) (n = 19)	(i) *Toxoascaris* 5(26.32)[b]	220±1.85[c] (100-400)	Nil
Total	5(26.32)	220 ± 1.85	
Hippopotami (*Hippopotami amphibous*) (n = 3)	(i) *Fasciola* 2(66.67)[a] (ii) *Dicrocoelium* 1(36.33)[b]	100±10.0[a] (50-150) 200±14.14[c] (200)	Nil Nil
Total	3(100)	300 ± 6.12	
Hartebeests (*Alcelaphus buselaphus*) (n = 9)	(i) *Strongyle* 2(100)[c]	75±8.66[b] (50-100)	(i) *H. contortus* (50 ± 2.5) (ii) *O. columbianum*(160.2±4.47)
Total	2(22.22)	75 ± 8.66	
Oribis (*Oribi oribi*) Nil	Nil	Nil	Nil

Keys:
a, b, c, d superscripted values differed significantly (P < 0.05)
S. papillosus = Strongyloides papillosus
H. contortus = Haemonchus contorus
T. colubriformis =Trichostrongylus colubriformis
O. columbianum = Oesophagostomum columbianum

Hymenolepis and Ascaris species mainly from carnivores and primates, may serve as a potential source for human infection.

The intensity of the infections showed that low to moderate egg counts were generally encountered among the various species except in a few cases. This might not be unconnected with the fact that large expanse of land available for roaming in the game reserve ensures a wider dispersal of infective parasitic stages and thus a reduction in the exposure rate of the animals to infection in contrast to very high egg counts often reported among captive wild animals due to build up of high concentrations of infective stages of helminthes within enclosures and cages (Nwosu, 1995; Mbaya *et al.,* 2006). Similarly, wild animals under free-roam especially primates have been reported to self medicate themselves with medicinal plants in their environment (Medicates naturalis) thereby, controlling helminthosis (Clayton and Wolf, 1995).

It can therefore be concluded that the wild animals in Yankari Game Reserve, Nigeria harboured a variety of helminth parasites of medical and veterinary importance. The prevalence of infection was moderate to high. Meanwhile, the intensity of infection (low-moderate) varied among animal species. It is therefore recommended that livestock be prevented from having contact with the wild animals or vice versa through regular patrols by game wardens. Similarly, tourists especially children, should avoid playing or ingesting dirt while on safari.

Impact

This manuscript finding shows how that the various helminths encountered among the wildlife in Yankari Game Reserve had high prevalence rates which can affect the in-situ conservation and productivity of these rare and almost endangered species. The recovery of the larvae of Haemonchus contortus alongside Trichostrongylus papillosus or Oesophagostamum columbianum among the Artiodactyla/Proboscidae and Ancylostoma species among the primates and carnivores might be of serious concern due to the haematophagus activity of these species in causing anaemia. Some of these helminths also had high intensities and may lead to future outbreaks in the in the event of stress.

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References

Agbede S M A and Yesufu M. 1982. Helminth parasites of wild carnivores in the zoological garden of Ibadan and the public health aspects. *Nigerian Veterinary Journal*, 11(2): 19 - 22.

Bamidele O and Ogunrinade A F. 1980. Helminth parasites of captive animals. *African Journal of Ecology*, 18(1): 265 - 266.

Barbosa A, Segovia J and Vergas J, 2005. Predictors of red fox (Vulpes vulpes). *Helminth Parasites Diversity,* 1(1): 3-14.

Boomer J, Horak I G and Knight M M. 1991. Parasites of South African Wildlife. IX. Helminths of Kudu (Tragelaphus strepsiceros) in the Eastern Cape Province. *Onderstepoort Journal of Veterinary Research*, 58(3): 203-204.

Christine D M, Muller-Graf. 1995. A coprological survey of intestinal parasites of wild lions (Panthera leo) in the Serengeti and Ngorongoro crater, Tanzania, East Africa. *The Journal of Parasitology*, 81(5): 812-814.

Clayton D H, Wolf N D. 1995. The adaptive significance of self mediation. *Trends in Ecology and Evolution*, 8: 60 - 63.

Crockett E O. 1983. A preliminary survey of some helminth parasites of game animals in Kainji Lake National Park: Borgu Sector, *MSc. Thesis, University of Ibadan*, Nigeria

Donald A, Donner A. 1988. The analysis of variance adjusted to chi-square tests when there is litter or herd correlation in a survey or field trials. Acts of Veterinary Science, 84: 490 - 492.

Enyinihi U K. 1972. Parasitic infection of animals in the University of Ibadan Zoo, *African Journal of*

Medical Sciences, 3: 283 - 293.

Hanseen J and Perry B. 1994. The Epidemiology, Diagnosis and Control of Helminth parasites of Ruminants, ILRAD, Kenya, pp: 79 - 92.

Isoun T T, Losos G T and Ikede B O. 1972. Diseases of zoo animals in Nigeria. *Journal of Wildlife Diseases,* 8: 335 - 339.

Lynn L R and Sussane M R. 2004. Parasites of bears: A review. *3rd International Conference on Bears. Paper 42*, pp: 411-430.

MAFF, 1977. Manual of Veterinary Parasitological Laboratory Techniques. Technical Bulletin No. 18. London, *Ministry of Agriculture, Fisheries and Foods*, pp: 129.

Mbaya A W and Nwosu C O. 2004. Outbreak of clinical toxocariasis among captive lions at the Sanda Kyarimi Park, Maiduguri, Nigeria. *Nigerian Journal of Experimental and Applied Biology*, 5: 239 - 242.

Mbaya A W and Aliyu M M. 2006. Mixed infection of Notoedres cati and Ancylostoma caninum among captive lions (Panthera leo) at the Sanda Kyarimi Park Maiduguri, Nigeria. *Sahel Journal of Veterinary Sciences*, 5(3): 55 - 58.

Mbaya A W, Nwosu C O, Aliyu M M, Ahmed T A, 2006. A comparative study of gastrointestinal parasites of captive and free-living wild animals in the semi-arid zone of North-eastern Nigeria. *Nigerian Journal of Experimental and Applied Biology*, 7(1): 185 - 193.

Mbaya A W. 2007. Studies on trypanosomosis in red fronted gazelles (Gazella rufifrons) in Nigeria. PhD Thesis, *University of Maiduguri,* Nigeria, pp: 45-47.

Mbaya A W, Aliyu M M. 2007. An outbreak of parasitic gastroenteritis among captive gazelles in Maiduguri. *In proceedings of the 44th Nigerian Veterinary Medical Association Conference*, pp: 273-277.

Mbaya A W, Aliyu M M, Nwosu C O, Ibrahim U I. 2008. Effect of Berenil® or Cymelarsan® on the biochemical changes in red fronted gazelles (Gazella rufifrons) experimentally infected with Trypanosoma brucei. Revue d'élevage et de medicine vétérinaire des pays tropicaux, 61(3-4): 169-175.

Mbaya A W, Nwosu C O and Ibrahim U I. 2009. Parasitic gastroenteritis (PGE) complex of domestic ruminants in Nigeria: *A review. Sahel Journal of Veterinary Sciences,* 8 (2): 57-68.

Meshram M D, Shirale S Y and Khillare K P. 2008. Incidence of helminthic infection in axis deer. *Veterinary World*, 1(1): 10-13.

Nwosu C O. 1995. Helminth parasites of captive wild animals in Borno state of Nigeria. *Tropical Veterinarian*, 13: 1 - 2.

Nwosu C O, Ogunrinade A F and Fagbemi B O. 2006. Effect of concurrent Trypanosoma brucei and Haemonchus contortus infection of red Sokoto (Maradi) goats. *Sahel Journal of Veterinary Sciences*, 5: 45 - 54.

Ogunji F A, Akinboade O A and Dipeolu O O. 1982. Gastrointestinal parasites and bacterial flora of wild animals in Borgu Game Reserve in Nigeria. *International Journal of Zoonosis,* 9: 62 - 64.

Sloss W M, Kemp L R. 1978. *Veterinary Clinical Parasitology*. Iowa, State University Press.

Soulsby E J L. 1982. Helminths, Arthropods and Protozoa of Domesticated animals. London, Bailliere Tindall and Company.

Young E, 1977. Capture and care of wild animals. Florida, Ralph Curtis books.

THE MONOGENEAN TREMATODE, GYRODACTYLUS, A MAJOR CONSTRAINT TO AFRICAN CATFISH (CLARIAS GARIEPINUS), PRODUCTION: A CASE STUDY OF SMALL SCALE FISH FARMS IN IBADAN, NIGERIA.

Tchokote E Y [1] and Olufemi B E[2]
[1]Dept Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
[2]Dept Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria

Abstract

The gill fluke of the Gyrodactylus spp was found to be the major parasite of Clarias gariepinus in cultured conditions in Ibadan, NIGERIA. This paper describes the results of a study carried out to screen the external parasitic burden of the African catfish Clarias gariepinus in its culture environment. A total number of one hundred and fifty fish samples were collected over a period of three months from small culture ponds and tanks in Ibadan town. A brief on management practice and disease history was obtained from every farm where samples were collected. Fish were weighed and measured for their lengths. Mean values and standard deviations were calculated. Fish was then euthanized; smears of skin, fins and gill tissues were prepared and analyzed. The following parasites were recovered from the skin; Costia in 2% of sampled fish, Trichodinella in 5%, Dactylogyrus in 7%, an unidentified parasite at 5% all these each in 8% of visited farms. The gill parasite Gyrodactylus was present in 31% of screened fish and in 25% of the visited farms it then represented 46% of the total parasites recovered in this study. This parasite occurred in high incidence, its effect on the host fish with regards to the type of pathology created may present an economic threat to the stability and growth on catfish industry with serious implications for food security. Measures need then to be taken with regards to capacity development in rapid fish disease diagnosis, prevention and control.

Key words: CLarias gariepinus, gill fluke, infestation

LE TREMATODE MONOGENE, GYRODACTYLUS, CONTRAINTE MAJEURE A LA PRODUCTION DU POISSON CHAT AFRICAIN (CLARIAS GARIEPINUS): UNE ETUDE TYPE SUR LES PETITES UNITES DE PRODUCTION PISCICOLE DANS LA VILLE D' IBADAN AU NIGERIA.

Resume

Le trématode monogène Gyrodactylus a eté le parasite le plus important du poisson chat Africain Clarias gariépinus en milieu de culture à Ibadan, au Nigeria. Ce papier présente les resultats d'une étude éffectuer pour déterminer le poids parasitic externe du poisson chat Africain en culture. Un total the cent cinquante échantillons de poissons a été collecté pendant une durée de trois mois des étangs et reservoirs de la ville D'Ibadan. Le mode de gestion et la santé était obtenus des differents lieux de production. Les poissons étaient pesés et leur taille mesurée, la moyenne des valeurs avec leurs écart-type enregistrés. Les poisons étaient ensuite euthanasiés, les preparations des tissues de la peau, des nageoires et des branchies éffectuées et analysées. Les parasites identifiés dans les preparations de la peau furent; le Costia, isolée de 2% de l'échantillon total, Trichodinella de 5%, Dactylogyrus de 7% et un parasite non identifié de 5% , tous retrouvés chacun dans 8% des unités de production piscicole visitées. La douve parasite des branchies de l'espèce Gyrodactylus était present dans 31% des poisons examinés et dans 25% des étangs/reservoirs et a representé 46% du total des parasites isolés dans cette étude. L'incidence eleve de cette douve et son effet pathologique sur son hôte presente une menace é conmique importante à la stabilité et la croissance dans la production industrielle du poisson chat Africain avec une repercussion dans l'auto-suffissance alimentaire. Ceci etant, des measures doivent être prises pour le development rapide des moyens de diagnostique, prevention et contrôle des maladies de poissons dans les confins des milieux de culture.

Mots clés: Clarias gariepinus, douve des branchies, infestation

Corresponding Author: eugeniyoungo@gmail.com

Introduction

In confines such as ponds and tanks with fish constantly coming in contact with each other, parasites transfer from fish to fish is inevitable. The ready availability of fish hosts results in high survival rate of juveniles from adult parasites or hatched from eggs and cysts deposited in the pond (Robert, 1992). This can lead to serious infections affecting many fishes. Parasitic diseases of fish may vary from simple sporadic infections which are self limiting; affecting a single or few individuals, to highly devastating outbreaks that may lead to serious economic loses. Such outbreaks in fish species, held in captivity under cultured conditions, in Africa often escape notice or are inadequately diagnosed (Paperna, 1980). Clarias gariepinus, though resistant to diseases and resilient to high density culture (Richter, 1976) with appreciable tolerance to environmental extremes (Haylor, 1991), has found to be affected with various parasitic infections both in the cultured and wild fish. Reports on parasitic infections and studies on parasitism in Clarias gariepinus in Nigeria has been made by researchers like Okaeme *et al* (1988), Bakare and Janson. (1992a).

The monogenean trematode has high affinity for the gill tissue, which is a very vital organ to the fish host since it is used for gaseous exchange; abnormalities in this organ will rapidly lead to asphyxiation and death.

(*) THE PARASITE WAS NOT IDENTIFIED TO THE LEVEL OF ITS GENUS

There is limited awareness on the occurrence of fish diseases in aquaculture systems in Africa and their impact on mortality rates. The problem is related to capacity fish disease diagnosis and prevention. Thus it may be difficult to compare cost of disease prevention to losses due to mortalities and inefficient feed conversion. Farmers chose to depopulate to reduce loss during periods of crisis. Some of the developing stages of the fish found sold on the market are not exclusively from wild harvest but from some fish ponds where owners have not been able to identify the problem and have opted for anticipated cropping to control disease spread and hence minimize loss. Capacity development and enhancing of existing knowledge on the common disease problems may pave way to implementation of routine preventive measures which will eventually promote a sustainable small scale fish production systems which are expected to bring maximum benefit to the farmers since the capital input in terms of establishment of the pond, running and maintenance cost would be minimized. Output from such farms have been determined to be significantly higher than that of medium and large scale system due to the biotechnology applied and running cost (Sarig, 1971). Nigeria being one of the largest producers of clarids in Africa (FAO, 1994); this development of capacity for disease diagnoses and control measures would be important to catfish production industry, protein supply and hence food security

Material and method

All 150 specimens collected from various ponds and tanks in Ibadan metropolis (see Table1) were examined within twenty four hours of collection. Skin was examined through its entire surface using magnifying hand lens (x10); fins were spread out and examined, eyes, oral cavity, operculum were kept open with the aid of a thumb forceps to view medial surfaces; gills were observed in-situ; external genitalia and anal region also were examined. Abnormalities such as skin discolorations, ulcers, presence of excessive mucus, increased or decreased ventilatory rates, opercula and fin lesions were recorded.

The weight, standard and total lengths of individual fish were carefully measured. Fish was then euthanized using a sharp blow on the head; smears of skin, fins and gill tissues were prepared.

The opercula were cut off and removed using scissors or scalpel blade. Gill arches were cut and removed. These were examined grossly for paleness, hemorrhages and other lesions. Samples of the gill lamellae were obtained from the arch near the base and mounted on a slide. A cover slip was applied and viewed under low power microscope for ciliates and flagellates, protozoan and metazoan parasites. Some of the

positive samples for parasitic infections were stained with giemsa solution and mounted into permanent slides.

Wet mount of skin smears was also made in the same manner as for the gills. In both cases distilled water was used for the preparation of the smears.

Results and interpretations

slolation of parasites from samples:
- Gill lesion, such as paleness, edematous gill, sloughing of gill lamellar tissue living a broom like structure, gross lesions resembling petechial hemorrhages were observed. The gill tissues that grossly showed petechiation were found microscopically to be heavily infested with the monogenean trematode, Gyrodactylus spp.

V. Gill smears:
The monogenean trematode, Gyrodactylus spp was seen to parasitize gill tissue. This view shows 2 parasites that picked the bluish giemsa stain on the gill filament. Four parasites can be seen in figure 2.

Table I: Location, management system and mode of feeding in visited farms.

Farm	Location	Water source	Mode of feeding	Disease history
1	University of Ibadan	Tap water	Intensive	None
2	University of Ibadan	Spring and rain	Semi-intensive	None
3	Amuro Olode	Spring	Semi-intensive	None
4	Amuro Olode	Spring	Semi-intensive	High mortality: suspected cannibalism.
5	Ajara village	Stream	Intensive	Broken head disease
6	Ajibode village	Well, rain, spring	Semi-intensive	None
7	Ojoo	Stream	Semi-intensive	None
8	Ojoo	Spring,rain,well	intensive	None
9	Mokola	Well	intensive	None
10	Bodija	Well	intensive	None

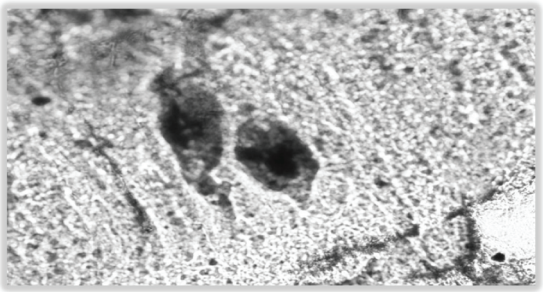


Figure 1: Gyrodactylus spp attached to the gill lamellae (x250).

Figure 2: Monogenean trematode as seen attached to the gill lamellae(x250).

VI. Histhopathology results.

Gill tissue of fish parasitized by the Gyrodactylus trematode. The figure 3 shows stunted filaments clubbed together with severe denudation, loss of villi (small arrows), focal rarefication of cartilaginous (big arrows) plates on the tips of the filaments and hypercellular lamina propriae.

VII. Representation of total parasites recovered:

Few of the visited farms were found to have parasitic problems. Of the affected fish (state number) the site most frequently parasitized was the skin (state the percentage of skin parasitized as compared to other areas of the body). However the gills were found to have more severe damage than the skin (percentage total parasites recovered from

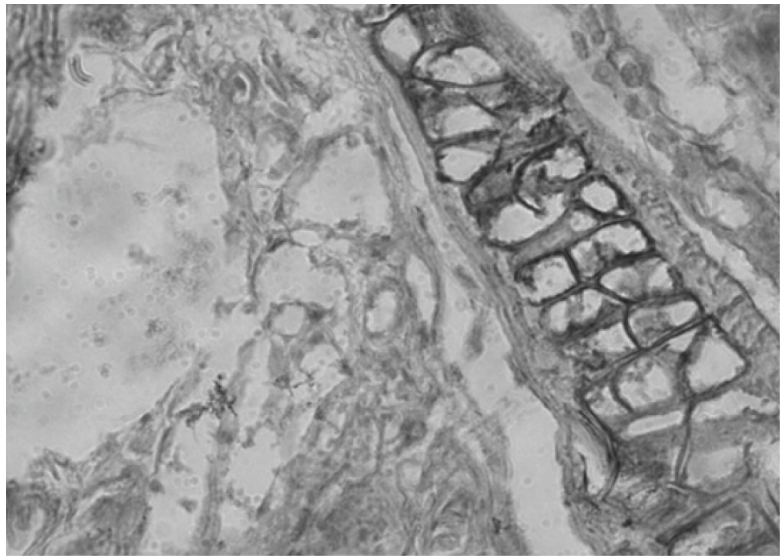
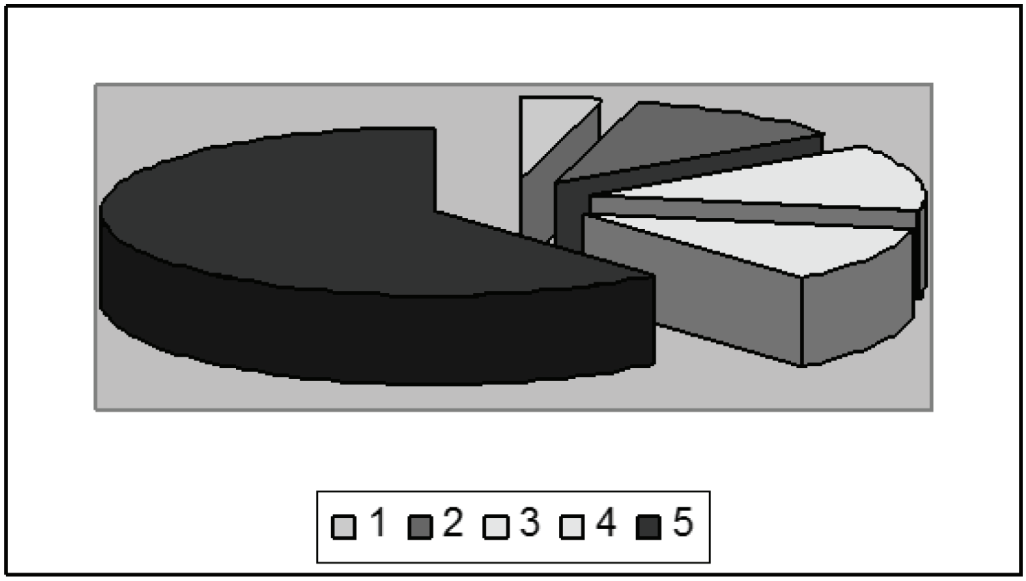


Figure 3: Histhopathology lesion in gill affected with gyrodactylus fluke (x1000)

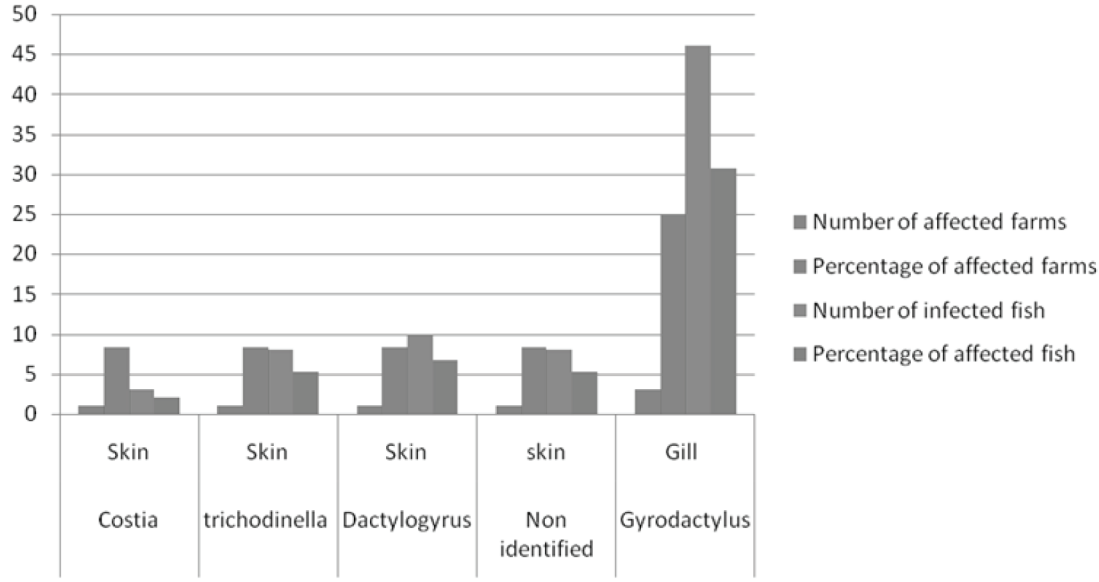
Table2: Total parasites recovered on screened fish.

Type of parasites	Sites	Number of affected farms	Percentage of affected farms	Number of infected fish	Percentage of affected fish
Costia	Skin	1	8.33	3	2
trichodinella	Skin	1	8.33	8	5.33
Dactylogyrus	Skin	1	8.33	10	6.66
Non identified	skin	1	8.33	8	5.33
No parasites seen	----	5	41.66	75	50
Gyrodactylus	Gill	3	25	46	30.66
Total		**12**	**100**	**150**	**100**



1 = Costia ; 2 = Trichodinella; 3 = Dactylogyrus; 4 = Non identified; 5 = Gyrodactylus

Figure 4: Percentage individual parasites recovered.



the skin is smaller(19.32%) than that of the gill (30.66%) as shown in the table below.

Conclusion

The productivity of fish farms can be very low if measures are not taken in disease control. Parasitic infections are found to present a great risk in the rapidly growing aquaculture fish production venture in Nigeria. Among isolated parasites the incidence of occurrence of monogenean trematode of the genus Gyrodactylus was found to be high amongst cultured fish species on fish farms in Ibadan. Without measures, viz capacity development in fish disease diagnosis, prevention and control, the parasite may present an economic threat to the stability and growth of catfish industry with serious implications for food security and employment in rural Nigeria. Because they are viviparous (produce live young), Gyrodactylus spp. can multiply very quickly, particularly in a closed system of tanks and ponds where water exchange is minimal. Presently, curative treatment against this parasite is said to be ineffective in most cases since sick fish may not respond to chemical agents used for treatment at the moment. Prophylactic treatment with a broad spectrum parasiticide may be suitable in treating this disease. An example of such treatment is administering formalin as a prolonged bath at 25 mg/L or a short-term bath or at 150-250 mg/L for 30 minutes. Potassium permanganate could also be used as a prolonged bath at a concentration of 2 mg/L or as a short-term bath (30 minutes) at a concentration of 10 mg/L. All fish should be carefully watched during chemical administration, if adverse reaction is observed, fish should be removed from the treatment tank at once and placed in clean water (UF/IFAS, 2003).

The monogenean trematode was found to parasitize 31% of the total fish sample examined, and 25% of visited ponds. Infected fish had a mean weight of between 130g and 220g, the range of actively growing population. Most of the samples were collected during cropping and as such one could say that anticipated depopulation was made to reduce loss caused by the disease. Other parasites (Trichodinella, Costia) isolated along with the gill fluke are potential threats to clarias production. Fish parasites could be transmitted to cultured ponds and tanks from hatcheries where fingerlings and juvenile fish are purchased. It is thus advised that farmers obtain their fingerlings from known sources or prophylactically treat juvenile/fingerling prior to stocking to minimize risk of infestation. Other factors that could predispose the fish host to severe infestation will include reduced resistance of the fish caused by stress, adverse

water quality such as high ammonia/nitrites levels, inappropriate pH or other toxins, severely altered protective properties of the cuticle. It is recommended that fish farmers maintain maximum biosecurity measures for optimal production.

References

Akinola, A. O. (1982). "Strategies for increasing fresh water fish production in the green revolution programme". *Proceeding of the 2nd Annual Conference of the Fisheries Society of Nigeria (FISON)*.

Bakare, T.A. Harris, L.P. and Janson, P.A. (1992a). "Host specificity and dispersal strategy in gyrodactylid monogeneans, with special reference to G. Salaris (platy-helminthes, Monogenea)". *Dis. Aqua*, 13: 63-74.

Clarias gariepinus (Burchell) caused by Gyrodactylus groschafti Ergens,1973. *Bull. Eur. Ass.Fish Pathol.,* 11: 82-85.

FAO,1994. Aquaculture production (1986-1992). Fisheries Circular No.815, Revision6. FAO Rome, 216pp.

FDA (2001): Parasites. Ch. 5. In Fish and Fishery Product Hazards and Controls Guidance, 3rd ed., p.65-72. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood ,Washington,DC.

Hoffman, G.L. (1970). Control and treatment of parasitic diseases of freshwater fishes.Fish Dis. Leafl. 28, 7pp.

Hoffman, G.L. and J. Lom (1967). Observation of Tripartella bursiformis. Trichodina nigra and a pathogenic Trichonid, Trichodina fultoni. Bull. Wildl. Dis. Associ. 3, 156-159.

Hogendoorn, H. (1981). Controlled propagation of the African catfish clarias lazera (C & V) reproductive biology and field experiment, Aquaculture 17, 323-333.

May, R.M. and Aderson, R.M. (1978). Regulation and stability of host-parasite population interactions. II destabilizing processes. *J. Anim. Ecol.* 47: 249-267.

Obiakezie, A.I. and Taege, M. (1991): Mortality in hatchery reared fry of the African catfish

Okaeme, A. N. et al (1988). Parasites and diseases of cultured fish of the lake Kainji area Nigeria. J. Fish Biology 32:479-481.

Olson, K. R. (1992): Blood and extracellular fluid volume regulation: Role of the rennin-angiotensin system, kallikrein-kinin system, and arthial natriuretic peptides. Pages 135-154 in Fish physiology volume xii, Part B: The cardiovascular system. San Diego: Academic Press.

Page, M. and Rowley, A.R. (1984): J. Fish Dis. 7, 339-353.

Paperna, I. (1980): Parasites and infections and diseases of fish in Africa in F.A.O. Word agricultural review (WAR) Cir.65.

Richter. C. J. J. and Husk, R. Vanden (1982). Effects or II-dosoxycorticosterone-acetate and carp ovaries of the African catfish C. gariepinus *J. Aqua.* (29). 53-66.

Robert, T. R. (1992): Systematic revision of the Old World freshwater fish family Notopteridae. Ichthyol. *Explor. Freshwaters* 2:361-383.

Sarig, S. (1971). The prevention and treatment of warm water fishes under subtropical conditions, with special emphasis on intensive fish farm, 127 pp. in S.F. Sineszko, and H.R. Azelrod (eds) Disease of fishes, Book 3, *T.F.H. Publications*, Inc., Jersey City.

GENETIC DIVERSITY OF INDIGENOUS CHICKENS IN CAMEROON

Fotsa J C
Institute of Agricultural Research for Development (IRAD), Mankon Specialized Research Station (SSRAD) Bamenda, Cameroon.

Summary

Over time, the adaptation to harsh environmental conditions by the indigenous chickens resulted in a huge genetic potential that goes beyond the mere short-term objectives of sources of income, food protein, and other relevant social practices. This type of poultry is challenged yearly by many epizooties that disseminate over 80% of the national flock size estimated to be 45 million. Gallus gallus remains the dominant species of the great family of Gallinaceans reared by 57% of women as the main stakeholders. Out of many poultry species, Gallus gallus is the only widely reared group in Cameroon and this study exemplifies the rich phenotypic potentials of its genetic diversity. Phenotypically, these chickens expressed feathered shank (gene PTI), crested head (gene CR), naked neck (gene NA), and frizzled (gene F) phenotypes representing 5.4%, 9.9%, 7%, and 0.8% of the population respectively. Their respective gene frequencies were 2.93%, 5%, 3.5%, and 0.4%. Sex-linked dwarf gene (DW*N) frequencies were 7.4% in males and 0.5% in females. Molecular studies by the use of microsatellites pointed out the expected (He) and observed (Ho) heterozygosity to vary from 0.617 to 0.634 and from 0.628 to 0.664, respectively. At the same time this study is made aware on the risks of introgression rates from 16% to 47% of improved breeds into the village chickens. In this condition, the gene pool of local chicken is experiencing a dangerous erosion of its original genetic diversity. The presence of relevant tropical genetic mutations can be associated with any poultry genetic improvement. Urgent conservation measures that will pave the way for subsequent progress are imperative.

Key words: Indigenous chicken, biodiversity, performance, introgression, conservation, Cameroon.

DIVERSITE GENETIQUE DU POULET INDIGENE AU CAMEROON

Résumé

Au fil des temps, l'adaptation des poules aux difficiles conditions environnementales locales a permis de modeler un patrimoine génétique indéniable qui va au-delà des simples objectifs de pourvoir à court terme une source de revenus et des besoins alimentaires en protéines animales ainsi que d'autres activités socioculturelle, religieuse et rituelles des populations. La poule locale est sujette à de nombreuses épizooties qui déciment annuellement plus de 80% du cheptel estimé à plus de 45 million de sujets. La poule Gallus gallus reste l'espèce dominante de la famille des gallinacées généralement élevée sous le contrôle des acteurs dont 57% sont de femmes. De nombreuses espèces disponibles, la Gallus gallus reste l'espèce la plus élevée au Cameroun. Cette étude illustre bien la riche potentialité de sa diversité génétique. Phénotypiquement ces poules expriment des caractères pattes emplumées (gène PTI), têtes huppées (gène CR), cou nu (gène NA) et frisé (gène F) représentant respectivement 5,4%, 9,9%, 7%, et 0,8% de la population avicole. Les fréquences géniques de ces mutations sont respectivement de 2,93%, 5%, 3,5% et 0,4%. La fréquence du gène du nanisme (DW*N) lié au sexe est de 7,4% chez les mâles et 0,5% chez les Femelles. Les études moléculaires chez les poules locales en utilisant les marqueurs microsatellites montrent des valeurs d'hétérozygotie attendue (He) et observée (Ho) variant respectivement de 0,617 à 0,634 et de 0,628 à 0,664. Cette étude a relevé des taux d'introgression variant de 16 à 47% des lignées améliorées dans les races locales ; ces taux entraînent ainsi une érosion génétique et par conséquent une perte de l'originalité de la diversité génétique chez les poules locales. La présence de mutations génétiques d'origine tropicale peut être associée à une amélioration génétique avicole. Des mesures urgentes de conservation sont impératives à l'effet d'ouvrir la voie à des progrès ultérieurs.

Mots clés: Poulet local, biodiversité, performance, introgression, Conservation, Cameroon

Corresponding author: fotsajc2002@yahoo.fr

Introduction

Indigenous chicken is known for its local adaptation to rearing environment and its resistance to some local diseases and perhaps emerging diseases. Adaptation of this type of poultry to harsh environmental conditions is probably due to genetic heritage acquired over years of domestication in a given environment (Fotsa and Poné, 2001). Estimated at more than 45 million in Cameroon (FAO, 2008), the national Cameroon poultry flock consists (Fotsa *et al.,* 2007) of about 70% of local chickens, 24% selected strains and 6% of other species known as unconventional poultry (guinea fowl, duck, geese, turkeys, pigeons) depending on ecological conditions. Moreover, because of its varied colorful feathers and that its meat and eggs are very appreciated by those who rear them, local poultry is a source of income and food animal proteins (Fotsa and Poné, 2001; Fotsa *et al.,* 2007). Rearing local poultry is a logical activity in the fight against poverty for the rural and peri-urban areas of Cameroon. Indeed, indigenous chicken is linked to socio-cultural, religious and ritual livelihoods for both urban and rural populations.

Progressively, reported emerging diseases continue to be a threat to poultry population, especially those reared intensively. With the resurgence of avian influenza or Bird flu in 2006, previous diseases such as Gumboro disease, Newcastle disease, chronic respiratory disease, fowl typhoid, fowl pox and parasitic diseases were reported to decimate over 80% of poultry flock (Ngou Ngoupayou, 1990; Ekue *et al.,* 2002). Faced with these disease challenges that regularly caused panic and wanton destruction of affected or suspected poultry flocks, it has become necessary to evaluate the genetic characteristics of indigenous chickens in Cameroon for better understanding of its genetic make-up that will be useful for its adequate future use.

Existing and introduced germplasms reared in Cameroon

Gallus (chickens) originated from the south-east Asia, northern India and Indonesian islands. It was domesticated in India about 5000 years ago (Cooke *et al.,* 2004). According to some authors, the presence of chickens in Africa is linked to early development of trade between India and East Africa (Carter, 1971) quoted by Crawford (1990).

The introduction of chickens in Africa is not well documented, but its production is traditionally rooted through migrations. It is the major domestic species and each household usually keeps a flock of 5 to 20 birds (Ngou Ngoupayou, 1990; Guèye, 1998; Fotsa *et al.,* 2007).

In Cameroon, Gallus gallus was initially alien but adapted over time by developing specific genetic traits to its environment. Commonly called "village chicken", it is known as Desi in Uganda, Baladi in Sudan, Fayoumi in Egypt; Nkoup nam in Cameroon (Ewondo and Bulu languages), Gup in Cameroon (Mankon language), Gouop Pebe in Cameroon (Bafoussam language), just to mention a few examples.

Chickens are reared in all agro-ecological zones of Cameroon and no real breed is stabilized. Quantitatively, Gallus is the dominant genus although scanty numbers of Anas (ducks), Numida (Guinea fowl), Meleagris (Turkey), Anser (Geese) and Columba (Pigeon) are found (Ngou Ngoupayou, 1990). Ekue et al. (2002) reported that in the North West Region of the western highlands of Cameroon, the genus Gallus ranked first in numbers (76.9%), followed by Anas (15.5%) and Columba (7.6%). These findings have been confirmed by Nfi *et al.,* (2011). Chicken population grew from 13 million in 1990 to over 25 million in 2001 and to almost 45 millions in 2008 (Ngou Ngoupayou, 1990; INS, 2001; FAO, 2006) because of their best potential for intensive farming and high productivity.

Phenotypically, the main genetic type of Gallus gallus showed a colorful feather pattern that varied in shape and size due to a great genetic variability. The population or genetic resources or strains also possess genes of tropical relevance such as naked neck (NA), dwarf (DW) and frizzle (F), which could be useful in genetic improvement programs. Other characteristics of the rural chicken are the presence of crested head (CR), feathered shanks (PTI), simple (R*N, P*N) to rose (R), walnut (R P) or pea comb (P*P, R*N), and

polydactyl (Po) phenotypes (Mafeni et al, 1997; Fotsa and Poné, 2001). Some of these traits are responsible for heat stress tolerance that has allowed local poultry to live and reproduce in adverse weather conditions like in the North and Far North of Cameroon with ambient temperature averaging 40°C (Table 1). Phenotypically, these chickens expressed feathered shank (gene PTI), crested head (gene CR), naked neck (gene NA), and frizzled (gene F) phenotypes representing 5.4%, 9.9%, 7%, and 0.8% of the population respectively. Their respective gene frequencies were 2.93%, 5%, 3.5%, and 0.4%. Sex-linked dwarf gene (DW*N) frequencies were 7.4% and 0.5% in males and females, respectively (Table 2). This diversity was confirmed with molecular tests (Fotsa et al. 2011).

Indigenous chickens' Performances

The growth rate of indigenous chickens at day-old, one week, five weeks and ten weeks-old are 32,7g, 40,04g; 199g; 583g respectively (Fotsa et Manjeli, 2001). These low performances could be explained by their genetic makeup since they are still low even when these chickens are bred in the intensive system (Yami, 1995; Fotsa et Manjeli, 2001; Bessadok et al. 2003). The recapitulation of the performances of the indigenous is presented in Table 3. When comparing the growth rate of the local chicken with that of the high yielding birds, it can be easily observed that the growth rate of the latter is 43.12% greater than that of the indigenous chicken even when they are bred in the same breeding condition. For the egg production, it was observed that some type of chickens harboring peculiar genetic characteristics could be favored (Ramlah et Kassim, 1992; Fotsa *et al.* 2011).

The danger of introgression on the poultry genetic resources in Cameroon

Molecular studies on some populations of local chickens in Cameroon showed observed heterozygosity (Ho) to range from 0.626 to 0.664, and an average number of alleles per locus of 7.09 (Fotsa, 2010) considerable genetic distance compared to the a German strain Dahlem Red (Mafeni, 1995).

Due of the low production observed in local chickens, crossbreeding with exotic strains have been attempted at rural level to improve their growth and egg production in Cameroon (Belot and Hardouin, 1981). This uncontrolled practice would lead to the disappearance of adaptive traits and the dilution of the gene pool and thus negatively influence the existing genetic diversity, especially as the genetic makeup of the imported strains is not disclosed. It was found introgression rates from 16% to 47% of improved breeds into the village chickens (Fotsa *et al.,* 2011). In this condition, the gene pool of local chicken is experiencing a dangerous erosion of genetic diversity for losing its originality. It showed that the majority of alleles in commercial populations can be found in the Cameroonian chickens for which the total number of alleles is 147 out of the 156 obtained using 22 microsatellite markers (Fotsa *et al.,* 2011). A high rate of introgression could cause some characters such as broodiness, the motherly character, resistance to some local diseases, the ability to feed themselves and fight predators to disappear and aggravate the risk of extinction of the breed. Furthermore, other important genes that could be used for poultry breeding programmes could be modified systematically over time and for good.

Proposals for Indigenous chicken's conservation in Cameroon

With reference to the current criteria of the World Union for Nature (IUCN) (IUCN, 2001) concerning the state of endangerment of a species and the list of species threatened with extinction (IUCN, 2004), Gallus gallus (village chicken) is not an endangered species. However, its vulnerability to outbreaks makes this threat to be real. Therefore, conservation measures should be considered within the scope of free range systems.

Such conservation measures include in-situ where efforts to inventory, collection and preservation of genetic material would be undertaken within the national territory, and ex-situ through gene banks and cryopreservation of genetic material and semen of these varieties (Verrier, 2006). Furthermore, blood samples of different genetic types should be collected to extract DNA for the enrichment of gene banks. These genes could be used in controlled

Table 1: Phenotypic frequency (%) of main feathering genes from pictures of 455 local chickens (Gallus gallus) observed in the forest zone of Cameroon

Feathering genes	Region					
	Centre		South		East	
	Female	Male	Female	Male	Female	Male
(E*E+E*R)[2]	32.15	24.28	36.99	60.72	38.75	58.82
B*B	8.57	19.00	12.33	25.00	16.25	17.65
PG*PG	7.14	0.00	8.22	0.00	11.25	0.00
S*S	5.00	16.00	12.33	25.00	16.25	55.88
MO*PI	5.71	4.00	1.37	0.00	5.00	5.88
I*I	10.00	12.00	9.59	7.14	10.00	20.59
C*N/C*N	0.00	1.00	1.37	0.00	0.00	2.94

E : Completely black ; ER : Extended black; B : Sex-linked Barred gene ; PG : Pattern gene ; S : Silver gene ; MOPI : Mottle ; I : Dominant white ; C*N (Recessive white [Crittenden et al. (1996) and Coquerelle (2000)];
1 : ddl = 2 ; [2] : Eumelanic phenotype; ** : $p<0.01$; NS : $P>0.05$
(Source: Fotsa et al. 2010)

Table 2: Gene frequency of visible genes observed during the genetic characterization of 455 local chickens in the forest zone of Cameroon

Phenotype	Region					
	Centre		South		East	
	Female	Male	Female	Male	Female	Male
n	94	333	42	92	49	141
Feathered shank 'PTI'	5.85	4.05		0.55	2.04	1.06
Crested head 'CR'	1.60	7.50	-	0.54	4.08	5.67
Heterozygous Naked neck 'NA'	7.98	4.36	-	1.64	1.02	1.42
Muff and beared 'MB'	-	-	-	-	-	0.71
Frizzle 'F'	-	0.15	-	-	1.02	1.40
Dwarf 'DW'	-	-	-	-	14.29	2.13

(Source: Fotsa et al. 2010)

introgression programs with the help of molecular markers with multi-locus probe (N'Dri, 2006). However, we shall keep in mind that Ex-situ method requires heavy financial resources which cannot be affordable by many developing countries (Singh *et al.,* 2011). Hence, it has been strongly suggested during the last INFPD/FAO e-conference held on February 2011 that research organizations, NGO's, private sectors and local farmers' community all should have to come under the same umbrella to implement a strategy for the sustainable preservation of the selected local breeds. To achieve the conservation/preservation goals, one must discourage all forms of dilution of the local gene pool by exotic blood (Singh *et al.,* 2011). Obviously the farmer's communities who are the key beneficiaries and who keep the birds in their houses should be included to keep the programme sustainable.

However, as pointed out during the last INFPD/FAO e-conference, conservation of a particular breed requires a complex management system which has to be based on scientific principles and which is a very costly affair. It needs proper planning, source of regular financing and follow-up of action plans. The first step would be to carry out a survey of the breeding tract. This should clarify the present status of the breeds, need and requirements of the farmers, their habits, management practices and types of breeds that exist locally. This type of information is rarely available in developing countries (Singh *et al.,* 2011).

Table 3: Recapitulation of local chickens' performances in Africa and some developing countries

Variable	Performance	Country	Authors
Adult weight (g)			
Females	1206	Tunisia	Bessadok et al . (2003)
	1050	Cameroon	Belot et Hardouin (1981) ; Ngou Ngoupayou (1990)
	1108-2020	Tanzania	Msoffe et al. (2001)
	1200-1400	Morocco	Benabdeljelil et Arfaoui (2001)
Males	1620	Tunisia	Bessadok et al . (2003)
	1140	Cameroon	Belot et Hardouin (1981) ; Ngou Ngoupayou (1990)
	1621-2915	Tanzania	Msoffe et al. (2001)
Males and Females	1020	Senegal	Missohou et al. (1998)
	1650-2200	South Africa	van Marle-Köster et Casey (2001)
Age at point of lay (days)	112-154	South Africa	van Marle-Köster et Casey (2001)
	162-166	Iraq	Al-Rawi et Al –Atari (2002)
	161	Senegal	Horst (1997)
	210-240	Belize et Guatemala	Mallia (1999)
	180	Guinea	Mourad et al. (1997)
	120	Mali	Ministère Français de la Coopération et du Développement (1991)
	140	Cameroon	Belot et Hardouin (1981)
	174	Morocco	Benabdeljelil et Arfaoui (2001)
	150	Côte d'Ivoire	Ministère Français de la Coopération et du Développement (1991) ;
	148.6	Tanzania	Hartmann et al. (2003)
Egg number	128 (Dandarawi)	Egypt	Horst (1991)
	141 (Fayoumi)	Egypt	Horst (1991)
	91	South Africa	van Marle-Köster et Casey (2001)
	40-60	Ethiopia	Yami (1995)
	31-40	Nigeria	Dafwang, (1989) ; Sonaiya et Olori (1990)
	60	Senegal	Missohou et al. (1998)
	78	Moroco	Benabdeljelil et Arfaoui (2001)
	40-80	Cameroon	Ngou Ngoupayou (1990)
	40-50	Belize et Guatemala	Mallia (1999)
	50	Sudan	Wilson (1979)
	127	Tunisia	Bessadok et al . (2003)
	30-120	Bangladesh	Paul et Huque (2000)

(Source: Fotsa et al. 2010)

Table 3: Recapitulation of local chickens' performances in Africa and some developing countries

Variable	Performance	Country	Authors
Annual mortality rate (%)	80	Guinea	Mourad et al. (1997)
	80-100	Cameroon	Belot and Hardouin (1981)
	77	Morocco	Benabdeljelil et Arfaoui (2001)
Number of brooding per year	2-3	Senegal	Guèye (1995)
	3.8	Guinea	Mourad et al. (1997)
	3	Mali, Burkina Faso, Niger	IEMVT-CIRAD (1989) ; Bantiéni et Modibo (2000)
	2-6 (average = 3)	Morroco	Benabdeljelil et Arfaoui (2001)
Egg number per brooding	10.5	Guinea	Mourad et al. (1997)
	6-19	Mali	Bantiéni é et Modibo (2000)
	12.4	Senegal	Missohou et al. (1998)
	8-20 (average = 14)	Morocco	Benabdeljelil et Arfaoui (2001)
	14	Cameroon	Tchoumboué et al. (2000)

(Source: Fotsa et al. 2010)

Nowadays, it would be necessary to establish a national conservatory for local chickens populations, a molecular biology laboratory for the development of a gene bank and equipment for cryopreservation of semen collected. All these preventive methods would likely restore Cameroon genetic diversity in case of disaster or epidemic of avian influenza type for instance.

Health wise, Cameroon poultry populations are annually under the threat of emerging diseases (Ekue *et al.,* 2002; Nfi *et al.,* 2011), although vaccination programs, treatment of infectious and parasitic diseases have been alleviating the death toll. These measures should continue in rural areas. Regarding the threat of avian influenza that emerged in 2006 in Cameroon; the local poultry could disappear if adequate preventive measures are not taken to protect these genetic materials.

Conclusion

The uncontrolled use of imported breeds for possible crossbreeding of the rural flocks could seriously jeopardize the existence of indigenous chickens apart from threatening the genetic diversity that might contribute to hopeful discoveries in the areas of immune resistance against important diseases currently bypassed. Indigenous poultry in Africa as a whole and in Cameroon in particular will still remain for decades to come; therefore, it will be a quick source of protein and will likely protect the population against malnutrition while improving their financial situation and satisfying their ritual needs. From all these above, indigenous chicken deserves special attention and conservation.

Impact

A lot of researches have been done in the field of genetic characterization of indigenous chicken in Cameroon in order to gather more information about this specie. With the available results and recommendations drawn from various studies, poultry scientists could exploit them and come out with high productive native chickens for the benefit of poultry stakeholders.

References

Belot J and Hardouin J, 1981. Observation sur l'élevage traditionnel du petit ruminant et de la volaille en milieu villageois au Cameroun. Rapport technique provisoire. In : Etude de l'élevage traditionnel Ovin et Avicole en milieu villageois. Projet de recherche au Cameroun. Institut de Médecine Tropicale «prince Léopold » Nationalestraat 155 b- 20, ANVERPEN, Belgique, pp 41.

Bessadok A, Khochlef I, El-Gazzah M, 2003. Etat des ressources génétiques de la population locale du

poulet en Tunisie, Tropicultura, 21 (4), 167- 172.

Cooke F, Dingle H, Hutchinson S, Mckey G, Schodde R, Tait N and Vogt R, 2004. The Encyclopedia of Animals: A complete visual guide. Frog City Press. San Francisco. USA. P. 254p

Crawford R, 1990. Poultry breeding and genetics, Elsevier, Amsterdam, 1123 p.

Ekue F N, Poné K D, Mafeni M J, Nfi A N and Njoya J, 2002. Survey of the traditional Poultry Production System in Bamenda Area Cameroon. In : Characteristics parameters of Family Poultry Production in Africa. Results of a FAO/IAEA Co-ordinated Research Programme IAEA, 15-25.

FAO, 2008. Revue du secteur Avicole: Document développé sur la base de la « Première évaluation du secteur avicole au Cameroun » éditeur : Division de la Production et de la Santé Animales de la FAO, Centre d'Urgence pour les Maladies Animales Transfrontalières, Unité de socio-économie, Production et Biodiversité, 54p

FAO. 2006. Première évaluation du Secteur avicole au Cameroon: Structure et importance du secteur avicole commercial et familiale pour une meilleure compréhension de l'enjeu de l'influenza aviaire. Projet OSR/GLO/MUL, Emergency assistance for the control and prevention of avian influenza, FAO, 48p

Fotsa J C and Poné D K, 2001. Study of some morphological characteristics of local chickens in North-West Cameroon. International Network for Family Poultry Development, 11 (2): 13-19.

Fotsa J C, Bordas A, Rognon X, Tixier-Boichard M, Pone Kamdem D and Manjeli Y, 2007b. Caractérisation des élevages et des poules locales et comparaison en station de leurs performances a celles d'une souche commerciale de type label au Cameroun. Journée de la Recherche Avicole, 7: 414-417.

Fotsa J C, Rognon X, Tixier-Boichard M, Ngou Ngoupayou J D, Pone Kamdem D, Manjeli Y and Bordas A, 2007. Exploitation de la poule villageoise dans la zone de forêt dense humide à pluviométrie bimodale du Cameroun. Bulletin de Santé et de Production Animales en Afrique, 55 : 59-73.

Fotsa J C and Manjeli Y, 2001. Analyse comparée des performances de croissance en claustration des poussins de souche locale, d'une lignée Jupiter et de leurs croisements F1. Annales des Sciences Agronomiques du Bénin, 2 (2): 181- 192.

Fotsa J C, 2010. Caractérisation des populations de poules locales au Cameroun. Edition Universitaire Européenne, ISBN-13: 978-613-1-54478-1. 308 p.

Fotsa J C, Rognon X, Tixier-Boichard M, G Coquerelle, Pone Kamdem D, Ngou Ngoupayou J D, Manjeli Y and Bordas A, 2010. Caractérisation Phénotypique des Populations de Poules Locales (Gallus Gallus) de la Zone Forestière Dense Humide à Pluviométrie Bimodale du Cameroun. Animal Genetic Resources information, 46:49-59.

Fotsa J C, Pone Kamdem D, Rognon X, Tixier-Boichard M, Manjeli Y and Bordas A, 2009. Influence de certaines mutations à effets visibles sur les performances pondérales et les mensurations corporelles chez la poule locale de la zone forestière dense humide, Bulletin de Santé et de Production Animales en Afrique 57 (3): 276-284.

Fotsa J C, Pone Kamdem D, Bordas A, Tixier-Boichard M and Rognon X, 2011. Assessment of the genetic diversity of Cameroon indigenous chickens by the use of microsatellites. Livestock Research for Rural Development. 23 (5), Article #118. Retrieved May 6, 2011, from http://www.lrrd.org/lrrd23/5/fots23118.htm.

Gueye E F, 1998. Village egg and fowl meat production in Africa. World's Poultry Science Journal, 54: 73-86.
INS-CAMEROUN, 2001. Institut National des Statistiques : Note annuelle sur le secteur primaire. Agriculture, Elevage, pêche. 30p.

Mafeni J M, 1995. Studies on Productivity, Immunocompetence of genetic diversity of naked neck and normal feathered Indigenous Cameroon and German Dahlem Red fowl and their crosses. Ph.D thesis. 111p.

Mafeni J Mase, Wimmers K and Horst P, 1997. Genetic diversity in indigenous Cameroon and German Dahlem Red fowl populations estimated from DNA fingerprints. Archiv für Tierzucht, 40: 581 – 589.

Nfi A N, Ekue F N, Pone D K, Mafeni J M, Nji-Mbua Colette and Tih Shiffe J, 2011. Serological survey of newcastle disease status in village chicken in the Bamenda highlands of Cameroon. Bulletin of Animal Health and Production in Africa, 59: 274-276.

N'dri A L, 2006. Etude des interactions entre génotype et environnement chez le poulet de chair et la poule pondeuse. Thèse de doctorat de l'INA-PG. 252p.

Ngou Ngoupayou J D, 1990. Country report on small holder rural poultry production in Cameroon. In: CTA Seminar proceedings on Small holder Rural Poultry production, 9-13 october 1990, Thessaloniki, Greece, 2: 39–41.

Ramlah A.H and Kassim H, 1992. Production performance of village chicken. In: Proceedings of the National IRPA Seminar (Agriculture Sector), (Eds Ho, Y.W., Vidyadaran M. K. Abdullah, N. Jainudeen, M.R. and Bahaman, A.R.), 2, 496-497.

Singh D P, Fotsa J C and Thieme O, 2011. Opportunities of poultry breeding programmes for family production in developing countries: The bird for the poor (Summary and Conclusions). International Network for Family Poultry Development/FAO/FIDA, www.fao.org/ag/againfo/themes/en/infpd/home.html.

UICN, 2001. Catégories et Critères de l'UICN pour la Liste Rouge: Version 3.1. Commission de la sauvegarde des espèces de l'UICN. UICN. Gland, Suisse et Cambridge, Royaume Uni. Ii + 32 p.

UICN, 2004. IUCN Red List of Threatened Species. www.redlist.org. Downloaded on 09/08/2005

Verrier E, 2006. Gestion et conservation des ressources génétiques animales. In: Verrier et Planchenault (Eds), les Ressources Génétiques à l'Orée des temps nouveaux, Bureau de Ressources Génétiques : 22-25.

Yami A. 1995. Poultry production in Ethiopia. World's Poultry Science Journal, 51: 197-201.

TESTICULAR MORPHOMETRY AND HISTOLOGY OF MALE WISTAR RATS AND GESTATIONAL PATTERN OF FEMALE WISTAR RATS TREATED WITH GRADED DOSAGES OF THE LEAVES' AQUEOUS EXTRACT OF SPONDIAS MOMBIN

Oloye A A[1], Oyeyemi M O[2], Ola-davies O E[3], Olurode S A[1] and Inamah O A[1].
[1]Department of Veterinary Public Health and Reproduction, College of Veterinary Medicine, Federal University of Agriculture Abeokuta.
[2]Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan.
[3]Department Of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University Of Ibadan.

Abstract

The effects of aqueous extract of Spondias mombin leaves on testicular characteristics and neonatal birth weights after oral treatment of male and female wistar rats with graded dosages were studied. Twenty-five female and male sexually matured wistar rats divided into four treatment groups B,C, D and E and control group(A) were used. Five untreated matured male were also used for mating. The treatment groups were given 200mg/kg, 400mg/kg, 600mg/kg and 800mg/kg respectively while distill water was served to the control group for 28 days. After, testicles were harvested and studied grossly and for histology while the females were served by sexually matured untreated male rats introduced at the last half of the oral treatment. The gross and histology studies showed normal conformation of all the testicles. There was no abnormality noticed in all. Weekly vaginal cytology done during treatment revealed that all the female rats cycled normally. Births were recorded in all groups within the average gestation range of 24-28 days. While group A (control group) had the highest average litter size of 13 followed by groups D and E with 9, group C and E had the highest average birth weights of approximately 5g. It is concluded that there was no antifertility consequence of aqueous spondias mombin on the male wistar rat but insipient infertility was noticed with lower dosages for the female but none with dosage as high as 800mg/kg.

Key words: Aqueous extract, Spondias mombin, testicular, neonatal, wistar rat

MORPHOMÉTRIE ET HISTOLOGIE DES TESTICULES DE RATS WISTAR MÂLES ET PROFIL GESTATIONNEL DES RATS WISTAR FEMELLES TRAITÉS AVEC DES DOSES PROGRESSIVES DE L'EXTRAIT AQUEUX DE FEUILLES DESPONDIAS MOMBIN

Resume

Les effets de l'extrait aqueux de feuilles de Spondias mombin sur les caractéristiques des testicules et les poids à la naissance des nouveau-nés après le traitement oral des rats Wistar mâles et femelles avec des doses progressives dudit extrait ont été étudiés. Vingt-cinq rats Wistar femelles et mâles sexuellement matures répartis en quatre groupes de traitement B, C, D et E et un groupe témoin (A) ont été utilisés. Cinq mâles matures non traités ont été utilisés pour l'accouplement. Les groupes traités ont reçu respectivement 200mg/kg, 400mg/kg, 600mg/kg et 800mg/kg, tandis que le groupe témoin a reçu de l'eau distillée pendant 28 jours. Par la suite, les testicules ont été prélevés et soumis à une étude microscopique et histologique. ; Les femelles ont accouplées par des rats mâles sexuellement matures non traités, on été introduites au traitement durant la dernière moitié de l'étude. Les études microscopiques et histologiques ont montré une conformation normale de tous les testicules. Aucun cas d'anomalie n'a été constaté. La

Corresponding author: drabimbola2002@yahoo.com

cytologie vaginale hebdomadaire faite pendant le traitement a révélé que toutes les femelles avaient des cycles normaux. Les naissances ont été enregistrées dans tous les groupes,après une période moyenne de gestation de 24-28 jours. Alors que le groupe A (groupe témoin) avait la plus grande portée moyenne de 13 ratons, suivi par les groupes D et E dont la portée était de 9, les groupes C et E avaient les poids les plus élevés à la naissance, soit environ 5g en moyenne. On a conclu que le spondias mombin aqueux ne causait pas d'infertilité chez le rat Wistar mâle ; un début d'infertilité a été remarqué avec des doses faibles pour la femelle, mais par contre une dose aussi élevée que 800mg/kg n'a pas produit un tel effet.

Mots-clés: Extrait aqueux, Spondias mombin, testiculaire, néonatal, rat Wistar

Introduction

Spondias mombin is a fruitiferous tree that belongs to the family Anacardiacae. It grows in the coastal areas and in the rain forest into a big tree of up to 15-22mm in height. It is readily common in Nigeria and is known as Iyeye in Yoruba language, Ngulungu in Igbo and Isada in Hausa. The plant is widely relied on for various herbal remedies for numerous conditions. It is a common midwife's remedy to help induce labour, reduce bleeding and pain during and after child birth, to bring on the flow of breast milk, and expulsion of placenta. Spondias mombin leaves are among the forages given to domestic animals in SouthEastern Nigeria (Ayoka *et al.,* 2008). The plant is given to expectant ruminant animals and those that delivered without the release of their placenta (Okwu and Ekeke, 2003) and also given for anti-helminthic activities (Ademola *et al.,* 2005). Saponin, found as one of its constituents, has oxytocytic effect (Offiah and Anyanwu, 1989); alkaloids have antispasmodic, analgesic and antibacterial activities (Corthout *et al.,* 1994). Flavanoids and other phenolic compounds also found in the plant have been associated with anti herpes, antioxidative, antiviral and anti ageing properties, (Corthout *et al.,* 1992).

Raji *et al.,* (2006) reported antifertility action of aqueous Spondia mombin bark extract in male wistar rat.

This study was carried out, using the wistar rat as a model, to investigate the effect the aqueous extract of the leaves can have on some reproductive potentials as the leaves are served animals for different medicinal purposes

Materials and Methods

Plant was collected identified and prepared into an extract at the University of Ibadan using standard method of extract preparation. Twenty-five female and twenty five male pubertal wistar rats divided into four treatment groups (five rats per group) B, C, D and E and control group(A) were used. They weighed 120g. The treatment groups were given 200mg/kg, 400mg/kg, 600mg/kg and 800mg/kg respectively while distill water was served to the control group for 28 days. The male were harvested of their testicles at the end of oral treatment and testicles were subjected to morphometry and histology. Vagina cytology of the female rats was done for two consecutive weeks to establish an estous cycle pattern. The female rats were thereafter served with untreated sexually matured male rats at the ratio of five female to one male. Identification of sperm plug in the vagina was taken as the day 0 of gestation. Gestation length, litter size, and live birth weight were measured. Student's t-test was used to analyse the data (Steele and Torrie 1996). The difference of means were considered significant at $p<0.05$. SPSS statistical package (version 16.0) was used.

Results

tThe testicular weights of the treated groups were not significantly different from the control group (Table 1). The gross and histology studies showed normal conformation of all the testicles (Figure 2). Average testicular length, width and epididymal length were not significantly different comparing all the groups and between right and left testes within each group (Table 2). There was no abnormality noticed in all. Weekly vaginal cytology done during treatment revealed that all the female rats cycled normally. Specific epithelial cells predominant and consistent with each stage

Table 1: Effect of aqueous extract of Spondias mombin on the testicular weight of treated wistar rats (±SD)

Group	Dosage (mg/kg)	Right testis (g)	Left testis (g)
A (Control)	Distilled water	0.9±0.15	0.9±0.20
B (200mg/kg)	200	1.1±0.10	1.0±0.06
C (400mg/kg)	400	1.1±0.10	1.0±010
D (600mg/kg)	600	0.9±0.06	1.0±0.12
E (800mg/kg)	800	1.2±0.10	1.1±0.00

Table 2: Effect of aqueous extract of Spondias mombin on the testicular length and width and epididymal length of treated wistar rats (±SD)

Group	Average Teticular length (cm)		Average Testicular Width(cm)		Average Epididymal Length (cm)	
	R	L	R	L	R	L
A	1.9±0.30	1.9±0.30	1.1±0.13	1.1±0.13	4.0±0.22	4.0±0.61
B	1.7±0.32	1.7±0.30	1.1±0.9	1.1±0.51	4.3 ±0.33	4.3±0.19
C	1.9±0.21	1.9±0.26	1.1±0.15	1.0±0.06	4.1±0.50	4.0±0.71
D	1.7± 0.06	1.7±0.06	1.1±0.13	1.1±0.13	3.5 ±0.56	3.7±0.45
E	1.7±0.10	1.8±0.10	1.2 ±0.22	1.1±0.10	4.1±0.10	4.2±0.20

Table 3: Effect of aqueous extract of Spondias mombin on the average gestation length, litter size and live birth weight of treated wistar rats (±SD)

Group	Number in group	Average Gestation Length	Average litter size	Average live birth weight (g)
A	5	24.7±2.52	12.7±4.73	4.6±0.57
B	5	24.7±4.16	4.7±0.577	4.9±0.94
C	5	28.5±7.05	6.5±2.08	4.8±0.57
D	5	22.3±1.53	8.0±4.50	5.7±1.01
E	5	24.7±7.23	8.7±0.577	4.74±0.48

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Rt test L	5	1.7800	.10954	.04899
Lft test L	5	1.8000	.10000	.04472

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Differencep	
					Lower	Upper
Rt test L	36.334	4	.000	1.78000	1.6440	1.9160
Lft test L	40.249	4	.000	1.80000	1.6758	1.9242

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Rt Test Width	5	1.1200	.04472	.02000
Lft Test Width	5	1.0800	.04472	.02000
Rt Epid Lgth	5	4.0000	.30000	.13416
Lft Epid Lgth	5	4.0400	.23022	.10296

One-Sample Test

	Test Value = 0					
	t	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Rt Test Width	56.000	4	.000	1.12000	1.0645	1.1755
Lft Test Width	54.000	4	.000	1.08000	1.0245	1.1355
Rt Epid Lgth	29.814	4	.000	4.00000	3.6275	4.3725
Lft Epid Lgth	39.240	4	.000	4.04000	3.7541	4.3259

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Gest Lgth	5	24.9800	2.22531	.99519
Litter Size	5	8.1200	2.98530	1.33507
Live BW	5	4.9400	.43932	.19647

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
GestL	25.101	4	.000	24.98000	22.2169	27.7431
LittS	6.082	4	.004	8.12000	4.4133	11.8267
LiveBW	25.144	4	.000	4.94000	4.3945	5.4855

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
WeightR	5	1.0400	.13416	.06000
WeightL	4	1.0250	.05000	.02500

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
WeightR	17.333	4	.000	1.04000	.8734	1.2066
WeightL	41.000	3	.000	1.02500	.9454	1.1046

ANOVA

WeightR	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.045	2	.023	1.700	.370
Within Groups	.027	2	.013		
Total	**.072**	**4**			

ANOVA

WeightR	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.015	2	.008	3.000	.250
Within Groups	.005	2	.002		
Total	**.020**	**4**			

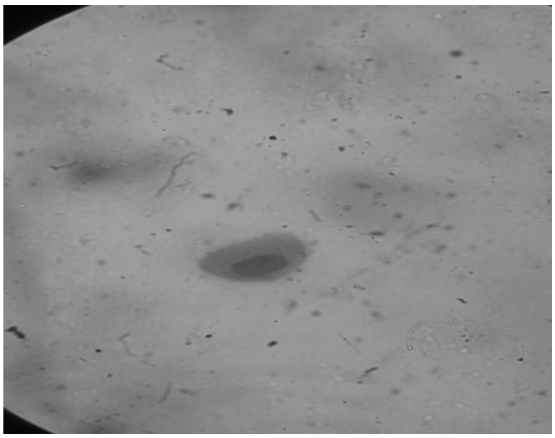


Figure 1: Parabasal epithelial cell indicative of active cycling

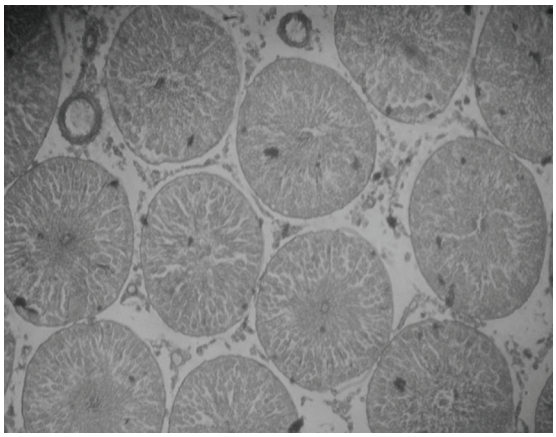


Figure 2: Normal Histological section of the testis of treated wistar rat

of estrous cycle were prominent (Figure 1). Births were recorded in all groups within the average gestation range of 24-28 days with Control group and Group B falling within the lower and upper extremes respectively (Table 3). While Group A (control group) had the highest average litter size of 13 followed by groups D and E with 9. Group C and E had the highest average birth weights of approximately 5g (Table 3). Group B animals appeared disadvantaged having the lowest Percentage conception and litter size.

Results

The testicular weights of the treated groups were not significantly different from the control group (Table 1). The gross and histology studies showed normal conformation of all the testicles (Figure 2). Average testicular length, width and epididymal length were not significantly different comparing all the groups and between right and left testes within each group (Table 2). There was no abnormality noticed in all. Weekly vaginal cytology done during treatment revealed that all the female rats cycled normally. Specific epithelial cells predominant and consistent with each stage of estrous cycle were prominent (Figure 1). Births were recorded in all groups within the average gestation range of 24-28 days with Control group and Group B falling within the lower and upper extremes respectively (Table 3). While Group A (control group) had the highest average litter size of 13 followed by groups D and E with 9. Group C and E had the

highest average birth weights of approximately 5g (Table 3). Group B animals appeared disadvantaged having the lowest Percentage conception and litter size.

Discussion and Conclusion

The undistrupted estous cycle give credence to the report of Nwude *et al.,* (2006) that the extract of Spondia mombin has no effect on the oestrogenic activity and by extension the oestrous cycle of the wistar rat. The unaffected male testicular characteristics after treatment is in consonance with Oloye *et al's* (2011) finding on the positive impact of the extract on the male spermiogram .The leaves served to animals for antihelminthic purpose is therefore safe in the male as it does not interfere with fertility. It is concluded that there is no antifertility consequence of aqueous spondias mombin on the male wistar rat but insipient infertility was noticed with dosages as low as 200mg for the non gravid female but none with dosage as high as 800mg/kg.

Impact

The use of natural products for enhancement of animal health can not be overemphasized. However it is needful also to ensure the preservation of the reproductive life of these animals in the course of using the natural products. This work has helped to ascertain the need for care in using Spondias mombin in pregravida animals.

Reference

Ademola I O, Fagbemi B O and Idowu S O. (2005): Anthelmintic activity of extract of Spondias mombin against gastrointestinal nematodes of sheep; studies in vitro and in vivo. *Tropical Animal Health and Production* 37 (3), 223 – 235.

Akubue P I, Mittal G C and Aguwa C N. 1983 Preliminary pharmacological study of some Nigerian medicinal plants. J. ethnopharmacol and; 8 (1) ; 53 – 63.

Ayeloja *et al* 2006 " Non- timber forest product marketing in Nigeria; A Case study of Osun state" *Educational Research and reviews*.pp 52-58 ISSN 1990-1839 OCLC 17318

Ayoka A O, Akomolafe R O, Akinsomisoye O S and Ukponmwan O E. (2008). Medicinal and Economic Value of Spondias mombin. African Journal of Biomedical Research (*Ibadan, Nigeria: Ibadan Biomedical Communications Group*) 11: pp.129–136.

Corhout J, Pieters L A, Claeys M, Vanden Berghe D A and Viletinck A J.(1992). Antiviral Caffeoyl; Esters from Spondias mombin Phytochemistry 31, 79

Corhout J, Pieters L A, Claeys M, Vanden Berghe D A and Viletinck A J.(1994). Antibacterial and Molliusciidal phenolic aid from Spondias Mombin. *Planta Medica*, 60, 460-463

Nwude N and Ibrahim M A. (1980).' Some plant used in traditional Veterinary medicine practice in Nig. *Presented at the 17th Annual congress of Nigerian Veterinary Medical Association in Zaria*..pg 28-31

Offiah V N and Anyanwu I I. (1989). Abortificient activity of an aqueous extract of Spondia mombin leaves. *J. Ethnopharmacol.* 26 (3): 317 - 320

Okwu D E and Ekeke O. (2003). Chemical composition of Spondia mombin plants. *J . sustain Agri. Environment* 6:140-147.

Oloye A A, Oyeyemi M O, Ola-davies O E and Innamah O A. (2011) Effect Of Aqueous Extract Of Spondias Mombin On The Spermiogram Of Wistar Rats *Bull. Anim. Health. Prod. Afr.* (2011). 59 : 95 - 99

EFFECTS OF TRAMADOL PREMEDICATION ON KETAMINE ANAESTHESIA IN YOUNG PIGS UNDERGOING SURGICAL CASTRATION

Ajadi R A[1]*, Okwelum N[2], Sonibare AO[1], Liebsch KR[3], Williams C E[3], Klein A L[3], Bennett M S[3], Kruse J T[3], and Gazal O S[2,3].

[1]Department of Veterinary Medicine and Surgery, Federal University of Agriculture, Abeokuta, Nigeria

[2]Department of Animal Physiology, Federal University of Agriculture, Abeokuta, Nigeria

[3]Department of Biological Sciences, St. Cloud State University, St. Cloud, Minnesota, USA.

Abstract

The effect of anaesthesia with tramadol -ketamine combination on behavioural, physiological and plasma cortisol changes was evaluated following surgical castration in pigs. Ten Large-white breed of pigs (9.8± 1.6 kg) were used in the study. The pigs were randomly assigned into two groups. Pigs in group one (KT) were anaesthetized with 5% Ketamine (20mg/kg) and 5% tramadol (3mg/kg), while pigs in group two (KS) were anaesthetized with ketamine and normal saline. Following anaesthesia, linear infiltration with 2% lignocaine was done before castration. Pain- associated behavioural changes (phonation, restlessness, struggling during surgery, and attitude to operation site) were evaluated during and up to two hours after surgery. Heart rates (HR), respiratory rates (RR) and rectal temperatures (RT) were recorded after anaesthesia, at onset of castration (time t= 0), and at 15 minutes interval over a period of 60 minutes. Blood was also obtained before, during and about 10 minutes after castration for the determination of plasma cortisol concentration. Behavioural changes were compared using Mann Whitney's test, while physiological parameters and plasma cortisol were compared using analysis of variance (ANOVA). Pain associated behavioural changes was significantly ($P< 0.05$) higher in KT than KS anaesthetized pigs. There was no significant difference in HR, RR and RT between and within treatments. Similarly, plasma cortisol concentration did not differ significantly between treatments. Both KT and KS combination failed to provide satisfactory intraoperative analgesia in grower pigs undergoing surgical castration.

Key Words: Ketamine, Tramadol, Castration, Pigs, Cortisol.

EFFETS DE L'ANESTHÉSIE AU TRAMADOL ET À KÉTAMINE CHEZ DE JEUNES PORCS SOUMIS À LA CASTRATION CHIRURGICALE

Résumé

L'effet de l'anesthésie par association du Tramadol et de la Kétamine sur le comportement, la physiologie et le taux du cortisol dans le plasma a été évalué après une castration chirurgicale de porcs. Dix porcs de race « Large White »(poids moyen de 9,8± 1,6 kg) ont été utilisés. Les porcs étaient répartis de façon aléatoire en deux groupes. Les porcs du premier groupe (KT) ont été anesthésiés avec une association de la kétamine à 5% (20mg/kg) et du tramadol à 5% (3mg/kg), tandis que les porcs du deuxième groupe (KS) ont été anesthésiés avec une association de la kétamine et d'une solution saline normale. Après l'anesthésie, une infiltration linéaire avec de la lignocaïne à2% a été effectuée avant la castration. Les changements de comportement associés à la douleur (phonation, nervosité, agitation pendant l'opération, et la réaction au toucher du site de l'opération) ont été évalués pendant l'opération et durant deux heures après l'intervention. La fréquence cardiaque (FC), la fréquence respiratoire (FR) et la température rectale (TR) ont été notées après l'anesthésie, au début de la castration (temps t = 0), et toutes les 15 minutes sur une période de 60 minutes. En outre, du sang a été prélevé avant, pendant et environ 10 minutes après la castration, afin de déterminer la teneur du cortisol dans le plasma. Les changements de comportement ont été comparés en utilisant le test de Mann-Whitney, tandis que les paramètres physiologiques et le taux de cortisol ont été comparés en utilisant l'analyse de la variance (ANOVA). Une valeur de $P< 0.05$

Corresponding Authors: ade_vsr@hotmail.com

a été considérée comme significative. Les changements de comportement associés à la douleur étaient nettement plus importants (P< 0,05) chez les porcs anesthésiés du groupe KT par rapport à ceux du groupe KS. On n'a pas noté de différence significative en ce qui concerne la FC, la FR et la TR entre et au sein des traitements.De même, la teneur du cortisol dans le plasma n'a pas significativement varié entre les traitements. Chez les deux groupes KT et KS,l'association de produits n'a pas pu fournir une analgésie peropératoire satisfaisante chez les porcs en croissancesoumis à une castration chirurgicale.

Mots-clés: Kétamine, Tramadol, Castration, Porcs, Cortisol.

Introduction

Male piglets are routinely castrated to prevent boar tainted meat and the difficulty of managing and handling intact boars. Till now, the most widely method used for piglet castration is surgery with or without used anesthesia (Fredriksen and Nafstad, 2006). Several events during surgical castration of piglets including scrotal incision, extraction of the testes, and severance of the spermatid cord are painful (Hay *et al.,* 2003, Prunier *et al.,* 2006). These have been shown to have detrimental effect on behaviour, physiology and health of the piglets (Taylor and Weary, 2000; Hay *et al.,* 2003,).

Surgical castration is performed under local or general anaesthesia because of pain and the welfare concerns associated with piglet castration. Subcutaneous and intra-testicular administration of lidocaine is the most commonly used anaesthetic technique for castration in young piglets (Fredriksen and Nafstad, 2006). Bupivacaine has been evaluated as an alternative to lidocaine because of its long duration of action. However, induction of analgesia was slower and the risk of post-operative infection was higher (Nyborg *et al.,* 2000). Inspite of the efficacy of intra-testicular lidocaine administration in young piglets, there might be a need for chemical agents in older piglets or grower pigs to facilitate effective restraint for handling and administration of the lidocaine for castration. Such agent must be able to provide both intra-operative and post-operative pain relief.

Ketamine is the most widely used anaesthetics in almost all species including pigs (Lin, 1996). This is so because the drug is considered to be relatively safe, as it generally causes minimal cardiovascular depression and it may actually stimulate cardiovascular function via its sympathomimetic effect (Wagner & Helleyer, 2000). In addition, ketamine can be administered intramuscularly as well as intravenously, making it practical for use in animals in which venous access is difficult. Tramadol on the other hand is an analgesic with mixed opioid and non-opioid activities(Garrido *et al.,* 2000). The non-opioid activity is mediated through alpha-2-agonist and serotonergic activity. Tramadol injection was reported to improve the efficacy of ketamine-xylazine anaesthesia in young pigs (Ajadi *et al.,* 2009).It is thus hypothesized that the combination of ketamine and tramadol will provide improved anaesthetic condition for grower pigs undergoing surgical castration. The aim of this study therefore was to evaluate the behavioural, physiological and plasma cortisol changes following premedication with tramadol in ketamine anaesthetized grower pigs undergoing surgical castration.

Materials & Methods

The protocol for this study was approved by the Ethical Review Committee of the College of Veterinary Medicine, University of Agriculture, Abeokuta, Ogun State, Nigeria. Ten Large-white male pigs with age ranging between 10-12 weeks and mean body weight of 9.8± 1.6 kg were used. The pigs were raised on a concrete floored pen and were fed on brewer's waste supplemented with palm kernel cake and bone meal. In addition, they were given ad-libitum access to water. Before the commencement of the study, the pigs were judged to be clinically healthy based on findings at complete physical examination.

The study used a simple randomized controlled design, the observers unaware of the drug regimen that was employed. Prior to

surgery, the pigs were starved overnight but were given access to water until the anaesthetic agent was about to be administered. The pigs were randomly assigned into two groups of five animals each. Pigs in group one (KT) were anaesthetized with 20mg/kg intramuscular injection of 5% Ketamine hydrochloride (Ketamine, Rotexmedica, Trittau, Germany) and 3mg/kg intramuscular injection of 5% tramadol (Amadol, Union Korea Pharma, Korea), while pigs in group two (KS) were anaesthetized with 5% Ketamine hydrochloride and equal volume of normal saline as in the KT group. All the pigs were pre-treated with 0.04 mg/kg intramuscular injection of atropine (Amopin, Yanzhou Xierkangtai Pharma, Yanzhou, China). Following anaesthesia, linear infiltration of 2% lignocaine (Xylocaine, Elcee laboratories, UK) at the scrotal groove was done to allow for castration. Following successful anaesthesia, the pigs were positioned in sternal recumbency and castration carried out using the scalpel method. The incision was later closed with a size 2/0 nylon monofilament. Pain- associated behavioural changes such as phonation, restlessness (non-purposeful movement associated with bleating), struggling during surgery, and attitude to operation site were evaluated during surgery and up to two hours after surgery and scores assigned. These changes were evaluated subjectively by a blinded observer.

Some physiological measurements such as heart rates, respiratory rates and rectal temperatures and were recorded. Hearts rates was determined with cardiac stethoscope, respiratory rates determined using abdominal excursion, and rectal temperatures using clinical thermometer. These were determined after anaesthesia, at onset of castration, (time t= 0) and at 15 minutes interval over a period of 60 minutes.

Blood was obtained from the caudal vena cava before castration, during castration and about 10 minutes after castration (time taken for completion of suture placement) for the determination of cortisol concentration in plasma. On each occasion, about 5mls of blood was obtained using a size 21 gauge needle. Cortisol concentration in plasma was determined using the Coat-A-Count Assay kit (Siemens Health Diagnostics, CA, USA), adapted and validated for caprine plasma (Kannan et al., 2001). Each sample was run in duplicate and the mean value obtained. Total binding was 51 percent and the non- specific binding was 1.1 percent. The sensitivity of the assay was 2ng/ml, while intra-assay coefficient of variation was 2.6%

Statistical Analysis:

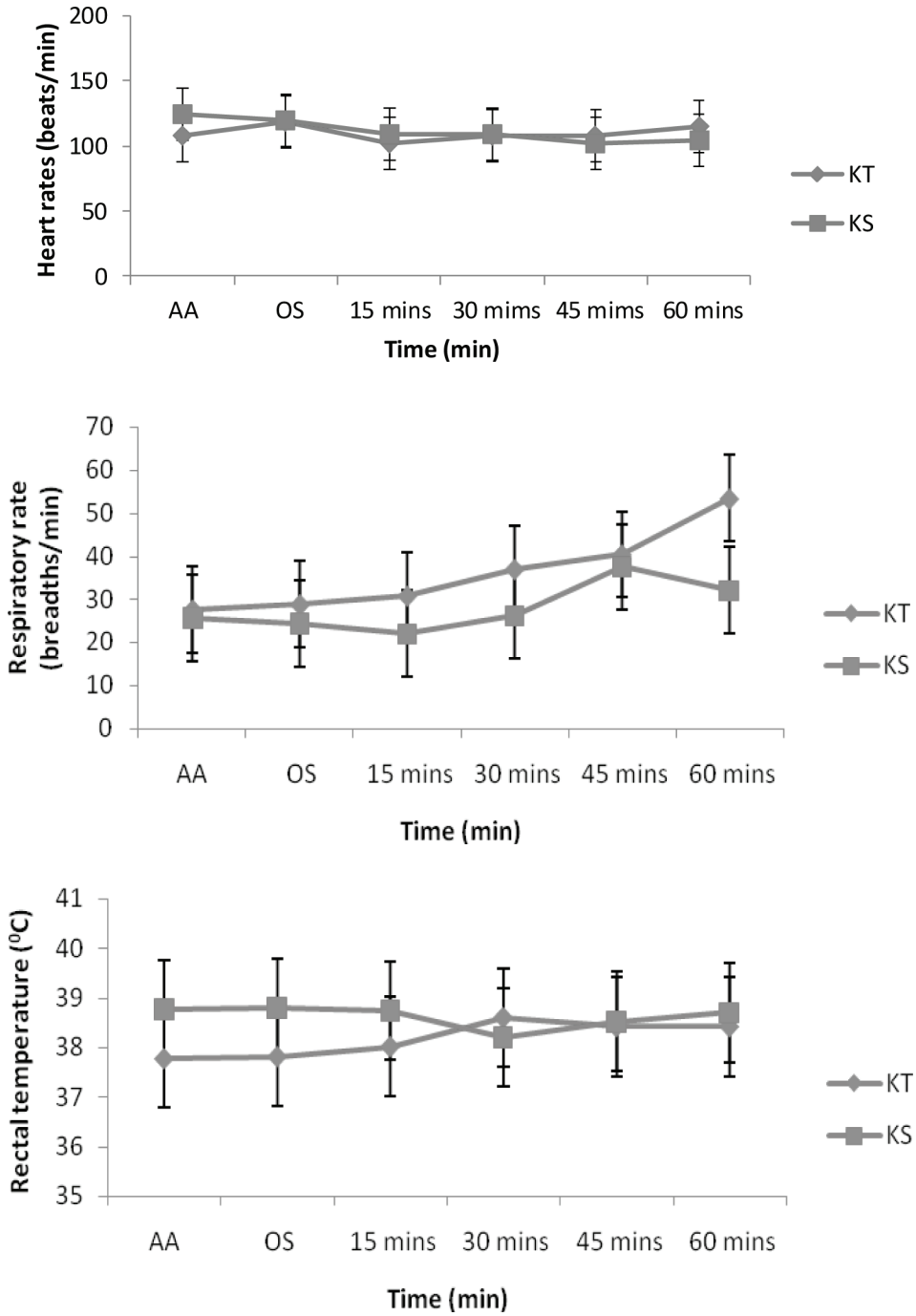
Data distribution was tested for normality by constructing frequency histograms of the data series. Differences in the behavioral scores between treatments were compared using Mann Whitney's test. Physiological parameters and concentration of cortisol in plasma were compared both for differences within and between treatments using analysis of variance (ANOVA) for repeated measures. Least square difference was used for post hoc analysis. A value of $P< 0.05$ was considered significant.

Results

Two of the pigs anaesthetized with ketamine and tramadol injection phonated while only one pig in the group that was anaesthetized with ketamine and saline phonated. Similarly, three pigs anaesthetized with ketamine and tramadol injection were observed to struggle during castration and were restless following castration, while only one pig from the group that received ketamine and saline struggle during castration and was restless following castration. Phonation was mild in both groups while pain perception was adjudged occasional in both groups. However, restlessness was scored as occurring always in the pigs that were anaesthetized with ketamine and tramadol injection while restless movement was occasional in the group that was anaesthetized with ketamine and saline injection.

There was no significant ($P>0.05$) differences in the heart rates, respiratory rates and rectal temperatures between and within treatments with ketamine -tramadol and ketamine- saline injection except at 60 minutes following castration when the respiratory rate was significantly ($P<0.05$) higher in

Fig. 1: Changes in heart rates (HR) respiratory rates (RR) and rectal temperatures (RT) in growing pigs either anaesthetized with ketamine-tramadol (KT, n=5) or ketamine-saline (KS, n=5) and subjected to surgical castration.



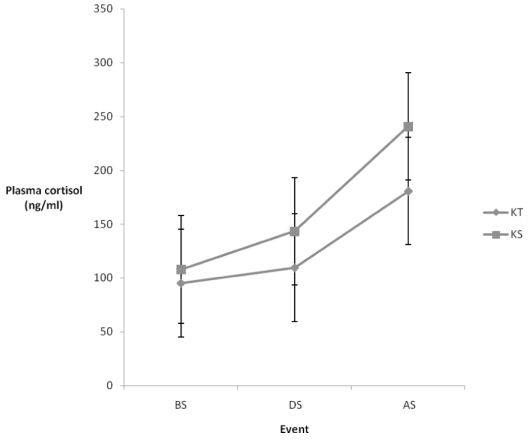
pigs anaesthetized with ketamine-tramadol injection compared with those anaesthetized with ketamine- saline injection (Fig. 1). Similarly, the concentration of cortisol in plasma did not differ significantly ($P< 0.05$) between and within treatments. However, the concentration of cortisol in plasma progressively increased from the value before castration up to the time after castration in both groups of pigs (Fig. 2).

Discussion

In this study, anaesthesia with ketamine-tramadol combination in pigs subjected to surgical castration was characterized by significantly higher pain associated behavioural signs compared with ketamine-saline combination. Both combinations also failed to suppress the concentration of cortisol in the plasma of pigs subjected to surgical castration. However, none of the combinations adversely affect the measured physiological parameters.

During castration, most piglets vocalize. Three types of vocalization have been identified during castration namely grunts, squeals and screams (Marx *et al.,* 2003). The number of screams was reported to be almost doubled in animals that were castrated without anaesthesia compared with those castrated with anaesthesia (Marx *et al.,* 2003). In this study, the only sound made by the pigs was adjudged to be grunting sound. This lower intensity of the sound made by the pigs might have been due to the ketamine anaesthesia used which tends to produce some degree of sedation in the pigs. However, addition of tramadol did not appear to influence the degree of sedation neither did it improve the degree of the antinociception. Tramadol unlike other opioids have been shown not to produce extra sedation when used in pigs thus resulting in a better recovery characteristic (Ajadi *et al.,* 2009).In general, it appeared that the degree of pain perception was higher in ketamine-tramadol combination than ketamine-saline combination. The exact reason for this observation is unknown as it would have been expected that pigs anaesthetized with ketamine-tramadol combination should have a lower pain score. It may also be that the signs observed may be more related to handling than to actual pain experienced during castration since plasma concentration of cortisol in both groups did not differ significantly to reflect the differences noted in the behavioural signs.

Fig. 2: Changes concentration of cortisol in plsama in growing pigs either anaesthetized with ketamine-tramadol (KT, n=5) or ketamine-saline (KS, n=5) and subjected to surgical castration.



Measurements of hormones following surgical castration in pigs have revealed the activation of the adrenal and sympathetic axes (Prunier *et al.,* 2005). Three- fold increase in the concentration of cortisol in plasma has been reported following castration in pigs (Prunier *et al.,* 2006). In this study, the concentration of cortisol in plasma steadily increased during surgery and up to about 15 minutes after surgery in both anaesthetic combinations suggesting that neither of the combination is able to suppress the activation of adrenal axes during castration in the pigs. This implies that both ketamine-tramadol and ketamine- saline combination is able to provide satisfactory stress relief in pigs undergoing surgical castration.

Finally, the addition of tramadol did not significantly change any of the physiological parameters measured in this study. This finding suggests that tramadol premedication causes no additional cardio-depressant effects in pigs neither does it activates the sympathetic axes in ketamine anaesthetized pigs subjected to surgical castration. This is similar to earlier findings in pigs anaesthetized with ketamine-xylazine combination (Ajadi *et al.,* 2009). The major advantage of tramadol use in this study therefore will be its lack of cardio-pulmonary depressant effect unlike other opioids such that pigs receiving the combination are more likely to maintain better intra-operative cardio-pulmonary functions. The clinical implication of this is that the anaesthesia related morbidity and mortality following castration in pigs is more likely to be lower with this combination compared with 28 percent mortality reported with the use of ketamine-xylazine-glyceryl guaiacolate combination (Prunier *et al.,* 2006).

In conclusion, addition of tramadol to

ketamine anaesthesia in young pigs subjected to surgical castration was characterized by pain associated behavioural signs and failed to suppress the cortisol concentration in plasma, although it produce a satisfactory cardio-pulmonary effect. Ketamine-tramadol combination did not appear satisfactory in abolishing the pain associated with castration in young pigs and will probably require another agent such as midazolam or ketamine to provide satisfactory pain relief.

References

Ajadi R A, Olusa T A, Smith O F, Ajibola E S, Adeleye O E, Adenubi O T, Makinde A F 2009. Tramadol improved the efficacy of ketamine-xylazine anaesthesia in young pigs. *Vet Anaesth & Analg* 36: 562-566

Fredriksen B, Nafstad O 2006. Surveyed attitudes, perceptions and practices in Norway regarding the use of local anaesthesia in piglet castration. *Res Vet Sci.* 81: 293-295

Garrido M J, Valle M, Campanero M A et al 2000. Modeling of the in vivo antinociceptive interaction between an opioid agonist (+) O-desmethyltramadol, and a monoamine reuptake inhibitor, (-) O-desmethyltramadol in rats. *J Pharmacol Exp Ther*. 95: 352-359

Hay M, Vulin A, Genin S, Sales P, Prunier A 2003. Assessment of pain induced by castration in piglets: behavioural and physiological responses over the subsequent 5 days. *Appl Anim Behav Sci*. 82: 201- 218.

Kannan G, Terrill T H, Kouakou B, Gazal O S, Gelaye S, Amoah E A, Samake S 2001. Transportation of goats: Effects on physiological stress response and live weight loss. *J Anim Sci* 78: 1450- 1457

Lin H C. 1996. Dissociative Anaesthetics. In Thurmon JC, Tranquilli WJ, Benson GJ eds. Lumb and Jones Veterinary Anesthesia 3rd Edition. Baltimore. *The Williams & Wilkins Co.* 241-296.

Marx G, Horn T, Thielebien J, Knubel B, von Borell E 2003. Analysis of pain –related vocalization in young pigs. *J Sound Vibration* 266: 687-698

Nyborg P Y, Sorig A, Lykkegaard K, Svendsen O 2000. Nociception after castration of juvenile pigs determined by quantitative estimation of c-Fos expressing neurons in the spinal cord dorsal horn. *Dansk Veterinaertidsskrift* 83: 16-17.

Prunier A, Moriez R, Mounier A M 2000. The use of plasma profile of cortisol and ACTH to assess the efficacy of local anaesthesia and preemptive analgesia in relieving pain at castration in piglets. *3rd International Workshop WAFL* - 05, 94.

Prunier A, Bonneau M, von Borell E, Cinotti S, Gunn M, Fredriksen B, Giersing M, Morton D B, Tuyttens F A M, Velarde A 2006. A review of the welfare consequences of surgical castration in piglets and the evaluation of non-surgical methods. *Animal Welfare* 15: 277-289.

Taylor A A, Weary DM 2006. Vocal responses of piglets to castration: identifying procedural sources of pain. *Appl Anim Behav Sci* 70: 17-26.

Wagner A E, Helleyer PW 2000. Survey of anesthetic techniques and concerns in private veterinary practice. *J Am Vet Med Assoc* 217: 1652 – 1657.

EFFECTS OF SUPERLIV® SUPPLEMENTATION IN FEED ON HAEMATOLOGICAL PARAMETERS OF POST PEAK SHIKA BROWN LAYERS.

Jibike G I[1], Arowolo R O A[2], Oladele, O. O[3], Agbato O[3] and Ohaebgulam O J[4].
[1]Department of Veterinary Physiology, Pharmacology & Biochemistry, University of Maiduguri, Nigeria. Authour for correspondence. gjibike@yahoo.com
[2]Department of Veterinary Pharmacology & Toxicology, University of Ibadan, Nigeria
[3]Animal Care Konsult Laboratories, Ogere, Ogun State, Nigeria.
[4]Department of Veterinary Anatomy, University of Maiduguri, Nigeria.

Abstract

The effects of Superliv®, a proprietary herbal feed additive, on some haematological parameters of laying hens were evaluated to determine the possible influence of the herbal feed supplement on the physiological status of laying birds in declining production phase of their life cycle. Fifty post peak Shika Brown layers were randomly assigned to five experimental groups (A-E) of 10 birds each. Group A birds were fed plain feed while groups B, C, D and E birds received feed supplemented with Superliv at the rate of 250g, 500g, 750g or 1000g per ton respectively over an experimental period of 12 weeks. They were monitored group wise, weekly, for some haematological parameters such as packed cell volume (PCV), red blood cell count (RBC), blood haemoglobin concentration (Hb), white blood cell count (WBC), erythrocyte indices and differential WBC counts. Superliv supplementation caused significant increases in PCV ($P<0.0001$), RBC ($P<0.0001$), Hb ($P<0.0001$) but no significant ($P>0.05$) changes in WBC, erythrocyte indices and differential WBC counts. The effects were most marked in the group that received 250g/ton of Superliv in feed. It is suggested that Superliv may induce production enhancement effects in post peak layers by acting as a haematenic. 250g/ton is recommended as the optimal inclusion rate for Superliv in layers' feed.

Key words: Feed additive, Herbal supplement, Haematology, Layers

EFFETS DU SUPPLEMENT ALIMENTAIRE SUPERLIV® SUR LES PARAMETRES HEMATOLOGIQUES DES PONDEUSES BRUNES SHIKA APRES LA PERIODE DE PRODUCTION MAXIMALE

Resume

Les effets de Superliv®, un additif alimentaire breveté à base de plantes, sur certains paramètres hématologiques de poules pondeuses ont été évalués afin de déterminer l'influence éventuelle de ce produit sur l'état physiologique des pondeuses après le pic de production. Cinquante pondeuses brunes Shika en période post-pic ont été réparties de façon aléatoire dans cinq groupes expérimentaux (A a E) de 10 oiseaux chacun. Les pondeuses du groupe A ont reçu une alimentation ordinaire, tandis que celles des groupes B, C, D et E ont reçu des aliments contenant le supplément Superliv® respectivement a des doses de 250g, 500g, 750g et 1000g par tonne respectivement, sur une période expérimentale de 12 semaines. Les pondeuses ont été suivies chaque semaine, afin d'étudier certains paramètres hématologiques tels que l'hématocrite (PCV), la numération érythrocytaire (RBC), la teneur en hémoglobine sanguine (Hb), la numération leucocytaire (WBC), les constantes érythrocytaires et les numérations leucocytaires différentielles. La supplémentation au Superliv® a provoqué des augmentations significatives de l'hématocrite ($P <0,0001$), de la numération érythrocitaire ($P <0,001$), de l'hémoglobine ($P <0,001$), mais elle n'a pas provoqué de changement significatif ($P> 0,05$) de lanumération leucocytaire, des indices érythrocytaires et des numérations leucocytaires différentielles. Les effets les plus marqués ont été observés dans le

Corresponding Author: gjibike@yahoo.com

groupe ayant reçu 250g/tonne de Superliv® dans son alimentation. Ces résultats permettent de penser que Superliv peut améliorer la production chez les pondeuses en phase post-pic en agissant comme un hématonique. La dose de 250g/tonne est recommandée comme taux de supplémentation optimale par Superliv dans l'alimentation des poules pondeuses.

Mots-clés: Additif alimentaire, Supplément à base de plantes, Haematologie, Pondeuses

Introduction

Feed additives are non critical, non nutrient inclusions in the diets of livestock, including poultry, in order to enhance production performance and feed conversion efficiency (Atteh, 2002). Non nutrient supplementation of livestock and poultry diets with growth promoter feed additives have been shown to elicit growth responses, enhance production target attainment and sustenance as well as promote overall physiological status of treated animals (Scott, 2009). Since the discovery in 1949 that the feeding of fermentation products of Streptomyces aureofaciens to pigs and poultry resulted in growth responses, the use of antibiotics as feed additives for growth and production promotion has become virtually universal (BSAS, 2009; Jukes and Williams, 1953). Growths promoting feed supplements, particularly antibiotic additives used in the poultry industry seem to have made the most beneficial impact on production enhancement, profitability and phenomenal growth of the industry (Mathews, 2001; Feighner and Daskevicz, 1987). However, despite their popularity and proven benefits in the poultry industry, the past three decades have witnessed a deluge of public health concerns arising from the use of sub-therapeutic levels of antibiotics as growth promoters in food animals, including poultry (BSAS, 2009; Butaye *et al.,* 1999). Several reports (WHO, 2002; FAO/WHO, 2001; Butaye *et al.,* 1999; Bates, 1997; Holmberg *et al.,* 1984) have implicated widespread use of antibiotics as feed additives in food animals in the development of drug resistant strains of bacteria with its attendant public health danger implications. These reports underscore the current global efforts to reduce the use of antimicrobials as feed additives in food animals to the barest minimum and search for alternatives to antimicrobials as growth promoter feed additives (Windisch *et al.,* 2008).

Phytogenic additives, including herbal feed additives have recently began to receive attention as comparatively potent but safer alternatives to antimicrobials as growth promoter additives in poultry and other livestock. The phytogenic feed additives comprise a wide variety of herbs, spices and products thereof which are mainly essential oils. Some promising reports have demonstrated experimental antimicrobial-like growth promoting effects of phytogenic essential oils (Smith-Palma et al., 1998; Si *et al.,* 2006), herbal feed additives (Namkung et al., 2004; Sarica *et al.,* 2005; Lien *et al.,* 2007) and antioxidant activities of plant derived essential oils (Wei and Shibamoto, 2007).

Superliv® is an Ayurvedic herbal preparation recently introduced into the feed additive industry and claimed to act as a liver stimulant when applied to various animal species in which it promotes productivity, reduces morbidity and mortality in treated flocks without toxic effects (Shivakumar *et al.,* 2005). Very recent report has demonstrated positive influences of Superliv feed supplementation on egg production and live weights of post peak Shika Brown layers (Jibike *et al.,* 2011). Superliv is a mix consisting of five related Ayurvedic herbs that enhance liver function, namely: Picorrhiza kuroa (Picorrhiza), Andrographis paniculata (Creat), Boerhauvia diffusa (Horse Purslane), Solanum nigrum (night shade) and Swertia chirata (Chirata). The present study attempts to explain the possible mechanism of action of Superliv by evaluating the effects of Superliv feed supplementation on some haematological parameters of post peak Shika Brown layers kept in the relatively hot North Eastern zone of Nigeria during a feeding period of 12 weeks.

Materials and Methods

Experimental Chicken

Fifty post peak Shika Brown layer chickens (Gallus gallus) that have been in lay for about 35 weeks were used. The hens were purchased from a reputable farm near Damaturu, Yobe State in the sahelian North Eastern region of Nigeria. On arrival, the hens were housed in standard battery cages, fed commercial layers' mash (Livestock Feeds®, Livestock Feeds Nig PLC) and allowed an acclimatization period of 2 weeks prior to commencement of the feed supplementation experiments. For the purpose of the experiments, the birds were randomly assigned to 5 treatment groups (A-E) of 10 birds each. They were housed in battery cages at the rate of 2 birds per cubicle. All the groups were housed under same roof and shared the same watering line but different feeding troughs. Group A birds were fed plain feed only while groups B, C, D and E received feed mixed with Superliv at the rate of 250g, 500g,750g or 1000g per ton of feed respectively, during the experimentation period of 12 weeks.

Experimental Feed

Commercial layers' mash (Livestock Feeds®, Nig. PLC) was used as basal feed. The approximate composition and ingredients of the basal feed have been reported (Jibike *et al.,* 2011). The feed was purchased in bulk and stored in off farm feed storage facility from which it was released in 25Kg bag units for rationing to the birds. Superliv was included in the feed of the respective treatment groups by mixing appropriate amounts of the herbal mixture in 25Kg units of feed as earlier described (Jibike *et al.,* 2011).

Superliv® Herbal mixture

Superliv feed additive was purchased from Animal Care Konsult Nigeria Ltd, the sole marketer of the Ayurvedic herbal preparation in Nigeria; as an amorphous greenish powder.

Phytochemical and Elemental analysis of Superliv herbal mixture

Superliv was subjected to elemental and phytochemical analysis using standard laboratory protocols. For qualitative phytochemistry, the herbal preparation was assayed for the presence of carbohydrates, tannins, anthraquinones, saponins, alkaloids and cardiac glycosides using standard methods (Michele *et al.,* 2003; Trease and Evans., 2002; Culer, 1982; Brain and Turner, 1975). Major trace elements including iron (Fe), copper (Cu), lead (Pb), cadmium (Cd), magnesium (Mg), calcium (Ca), sodium (Na) and potassium (K) were evaluated by Ashing and Atomic Absorption Spectrophotometry (Bolann *et al.,* 2007; Clegg *et al.,* 1981).

Experimental Protocol

Blood samples were collected from five birds in each group on alternate weeks, through the wing vein, for haematological evaluations. About 5ml of blood was collected from each bird using sterile 21gauge hypodermic needle and 5ml syringe. 2ml was discharged into heparinized universal sample bottles for haematological determinations while the rest was allowed to clot and yield serum samples for serology. The heparinized blood sample was used to determine: packed cell volume (PCV) by microhaematocrit method, blood haemoglobin concentration by cyanomethaemoglobin method (Jain, 1993), red blood cell (RBC) and white blood cell (WBC) counts using Neubauer counting chamber and avian RBC diluting fluid (Hewitt, 1984). Erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were determined by standard procedures while differential WBC counts were evaluated using Giemsa stained thin blood smears as previously described (Jain, 1993).

Statistical Analysis

Data collected are presented as tables of weekly group means (± SD). Two way analysis of variance (ANOVA) was used for statistical analysis of data with confidence limits set at 95%. Overall means separation was by standard procedures applied by statistical software used (GraphPad, 2000).

Results

Table 1 shows the results of phytochemical analysis while Table 2 represents determined elemental profile of Superliv mixture expressed in percentage of dry weight.

Table 1: Results of phytochemical analysis of superliv® herbal Feed additive and detected component compounds

COMPOUND	TEST	RESULT*	
1. CARBOHYDRATES:		General	Molisch
Positive (+)			
Monosaccharides	Barfoed's	Negative (-)	
Reducing Sugars	Fehling's	Positive (+)	
Combined red. Sugars	Fehling's	Positive (+)	
Ketoses	Salivanoff's	Positive (+)	
Pentoses	Hydrochloric acid	Negative (-)	
Soluble starch	Sulphoric acid	Positive (+)	
2. TANNINS:			
	Ferric chloride	Positive (+)	
	Lead acetate	Positive (+)	
	Hydrochloric acid	Negative (-)	
3. ANTHRAQUINONE:			
	Borntrager's	Negative (-)	
4. CARDIAC GLYCOSIDES:			
	Salkowski's	Positive (+)	
	Liebermann-Burchard's	Negative (-)	
Terpenoids	Sulphoric acid	Negative (-)	
Cardenolides	Keller-Killiani	Negative (-)	
5. SAPONINS GLYCOSIDES:			
	Frothing test	Positive (+)	
	Fehling's precipitation	Positive (+)	
6. FLAVONOIDS:			
	Shinoda's	Positive (+)	
	Ferric chloride	Positive (+)	
	Lead ethanoate	Positive (+)	
	Sodium Hydroxide	Negative (-)	
7. ALKALOIDS:			
	Dragendorff's reagent	Positive (+)	
	Mayer's reagent	Negative (-)	

*(+) present, (-) not detected

Tables 3, 4, 5 and 6 demonstrate the weekly and overall mean PCV, RBC count, Hb or WBC count respectively, of the various groups of layers assessed. Table 7 shows the overall mean values of erythrocyte indices and differential WBC counts.

Discussion

Results of the present studies demonstrate positive effects of Superliv herbal feed additive on the red cell parameters of post peak Shika Brown layers reared in sahelian region of Nigeria. Superliv feed supplementation as employed in this study caused significant increases in overall mean PCV, RBC and Hb but did not produce significant changes in overall mean WBC and differential WBC counts as well as erythrocyte indices. The increases in the overall mean PCV, RBC and Hb as observed among the hens that received Superliv

Table 2: Results of preliminary elemental analysis of superliv® Herbal feed additive powder

ELEMENT	QUANTITY DETECTED (g/ton)	% COMPOSITION
Zinc (Zn)	ND*	NIL
Iron (Fe)	0.6759	0.000068%
Copper (Cu)	ND	NIL
Lead (Pb)	ND	NIL
Calcium (Ca)	0.4235	0.000042%
Magnesium (Mg)	0.2118	0.000021%
Cadmium (Cd)	ND	NIL
Sodium (Na)	8.2149	0.00082%
Potassium (K)	4.3849	0.00044%

Table 3: Mean (+sd) weekly packed cell volume (pcv %) of groups of shika brown layers fed plain feed(a) or feed containing 250g/ton(b), 500g/ton(c), 750g/ton(d) or 1000g/ton(e) of superliv® as feed additive during a study period of 12 weeks

Weeks of feeding	GROUPS				
	A	B	C	D	E
0	24.00 + 4.18	23.80 + 3.49	23.20 + 3.11	25.20 + 8.35	23.00 + 4.24
1	22.00 + 2.35	26.60 + 1.67	26.80 + 1.30	23.40 + 2.88	26.40 + 1.82
2	22.00 + 4.36	23.00 + 2.94	24.50 + 3.70	25.80 + 4.44	22.50 + 5.80
3	27.67 + 1.53	28.50 + 2.38	30.00 + 7.31	33.75 + 2.63	26.40 + 4.16
4	22.75 + 3.10	28.25 + 1.71	27.75 + 0.96	32.75 + 3.86	26.40 + 1.14
5	22.60 + 2.70	26.60 + 3.21	24.20 + 3.90	25.20 + 4.82	25.50 + 2.81
6	25.40 + 1.14	29.00 + 4.30	26.60 + 3.71	28.40 + 2.19	28.20 + 2.17
8	24.33 + 0.58	29.67 + 2.52	29.00 + 2.45	29.25 + 2.22	28.00 + 1.15
9	25.00 + 1.58	27.40 + 3.58	26.40 + 2.51	26.40 + 0.89	26.80 + 1.64
10	25.60 + 2.07	26.20 + 0.84	26.80 + 0.84	27.00 + 1.22	26.60 + 0.89
11	25.80 + 2.17	26.40 + 1.34	25.20 + 0.84	26.80 + 0.84	25.60 + 2.19
***Overall**	**24.29^{a} + 1.82**	**26.86^{b} + 2.06**	**26.40^{b} + 2.05**	**27.63^{b} + 3.20**	**25.95^{ab} +1.79**

*Overall means with different superscript(s) differ significantly ($P < 0.0001$)

supplementation in diets may present the herbal feed additive as a haematenic agent, probably exerting positive influences on erythropoiesis. Similar observations of increases in RBC counts has been reported in respect of dietary inclusions of phenyl-lactic acid, a phytogenic agent, as feed additive in laying hens (Wang *et al.,* 2009). These observations are consistent with previous reports involving grower cockerels whose feed was supplemented with stressroak, an Ayurvedic herbal feed additive (Oyegbami *et al,* 2008). The increases in red cell parameters observed in the current study may be related to the elemental iron (Fe) content of Superliv as demonstrated in Table 2. Fe is a well known component of haematenic preparations that act as blood boosters (Morrison et al, 1977). The group that received the highest inclusion level of Superliv (1000g/ton) in feed showed marginally lower overall mean RBC and PCV values relative to other supplemented groups. This is rather surprising and may indicate possible toxic effects of Superliv at that inclusion rate. Superliv contains saponins (Table 1) that are capable of haemolysing red blood cells (Francis *et al.,* 2002) and at such high inclusion level, saponin concentration in the plasma may have risen to toxic levels as to cause some degree of haemolysis. The marginally higher overall mean Hb even when RBC and PCV values recorded were lower in this group may support the possibility of

Table 4: Mean (+sd) red blood cell (rbc) count (x1012/l) of groups of shika brown layers fed plain feed(a) or feed containing 250g/ton(b), 500g/ton(c), 750g/ton(d) or 1000g/ton(e) of superliv® as feed additive during a study period of 12 weeks

Weeks of Feeding	GROUPS				
	A	B	C	D	E
0	2.17 + 0.24	2.61 + 0.56	3.01 + 0.74	2.41 + 0.46	2.32 + 0.46
1	2.11 + 0.15	2.61 + 0.32	2.29 + 0.22	2.15 + 0.33	2.42 + 0.55
2	2.65 + 0.43	2.53 + 0.69	2.61 + 0.17	2.65 + 0.33	2.42 + 0.48
3	2.94 + 0.48	3.58 + 0.53	3.31 + 1.02	3.97 + 0.69	2.68 + 0.52
4	2.43 + 0.50	3.07 + 0.52	2.89 + 0.21	4.17 + 0.47	2.71 + 0.21
5	2.43 + 0.62	2.77 + 0.13	2.59 + 0.12	2.47 + 0.69	2.60 + 0.43
6	2.34 + 0.13	3.28 + 0.68	2.53 + 0.13	3.02 + 0.20	3.25 + 0.57
8	2.70 + 0.18	3.69 + 0.53	3.13 + 0.55	3.94 + 0.45	3.49 + 0.24
9	2.61 + 0.32	3.48 + 0.33	3.11 + 0.72	2.97 + 0.40	2.96 + 0.68
10	2.94 + 0.57	3.49 + 0.13	2.94 + 0.28	3.30 + 0.23	3.32 + 0.28
11	2.57 + 0.45	3.07 + 0.45	2.51 + 0.35	2.96 + 0.45	2.33 + 0.22
***OVERALL**	**2.54^{a} + 0.27**	**3.11^{b} + 0.43**	**2.81^{ab} + 0.32**	**3.09^{b} + 0.68**	**2.77^{ab} + 0.42**

*Overall means with different superscript(s) differ significantly (P < 0.0003)

Table 5: Mean (+sd) blood haemoglobin concentration (g/dl) of groups of shika brown layers fed plain feed(a) or feed containing 250g/ton(b), 500g/ton(c), 750g/ton(d) or 1000g/ton(e) of superliv® as feed additive during a study period of 12 weeks

Weeks of Feeding	GROUPS				
	A	B	C	D	E
0	7.00 + 0.28	7.43 + 0.78	7.54 + 0.92	8.05 + 2.12	7.28. + 1.02
1	7.44 + 0.54	8.84 + 0.65	9.08 + 0.85	7.72 + 1.24	8.56 + 0.94
2	8.83 + 1.00	9.45 + 1.50	9.68 + 1.69	9.84 + 0.47	9.43 + 1.83
3	8.80 + 1.06	9.18 + 0.48	10.58 + 1.43	11.10 + 0.55	7.90 + 1.26
4	7.18 + 0.77	9.10 + 0.87	8.45 + 0.43	10.13 + 1.21	8.44 + 1.36
5	7.28 + 1.03	8.10 + 1.83	7.68 + 1.79	8.08 + 1.17	7.02 + 0.97
6	8.48 + 0.73	9.70 + 0.69	9.04 + 0.74	9.56 + 0.67	9.60 + 0.60
8	7.27 + 0.31	8.20 + 1.04	7.90 + 0.77	8.40 + 0.73	7.80 + 0.69
9	8.88 + 0.52	9.64 + 0.17	9.50 + 0.33	9.36 + 0.17	9.52 + 0.23
10	9.28 + 0.97	9.64 + 0.17	9.64 + 0.33	9.40 + 0.20	9.40 + 0.24
11	9.04 + 0.38	9.04 + 0.30	8.74 + 0.42	9.16 + 0.46	9.00 + 0.58
***OVERALL**	**8.13^{a} + 0.89**	**8.94^{b} + 0.74**	**8.89^{b} + 0.95**	**9.16^{b} + 1.02**	**8.54^{ab} + 0.93**

*Overall means with different superscript(s) differ significantly (P < 0.0004)

a haemolytic process. Other workers have reported similar marginal decreases in RBC, PCV or both associated with exposure of chicken to phytogenic preparations having considerable saponin content (Odesanmi *et al.,* 2010; Olufemi *et al.,* 2003; Asif and Wahid, 2003).

That the erythrocyte indices remained unchanged and within normal range, may reflect absence of any form of haemopathology in the test and control birds, since erythrocyte indices changes are mostly associated with disease processes (Jain, 1993). Other workers have reported no changes in erythrocyte indices following exposure of birds to phytogenic preparations (Nworgu *et al.,* 2007). The overall mean values of total and differential WBC counts of control and Superliv supplemented

birds are statistically similar though the test groups showed marginally higher values. This is in contrast to previous reports (Oyagbemi *et al.,* 2008., Agarwal *et al.,* 1999) of increases in WBC count of chicken following exposure to herbal preparations through feed or water. Insignificant changes in WBC counts observed in the current study may reflect the healthy status of both control and Superliv supplemented birds. However, the overall mean WBC values of all supplemented groups are marginally higher than that of plain feed fed control birds; indicating subtle positive effects of Superliv on the WBC status in a manner

Table 6: Mean (+sd) white blood cell (wbc) count (x109/l) of groups of shika brown layers fed plain feed(a) or feed containing 250g/ton(b), 500g/ton(c),750g/ton(d) or 1000g/ton(e) of superliv® as feed additive during a study period of 12 weeks

Weeks of Feeding	GROUPS				
	A	**B**	**C**	**D**	**E**
0	14.70 + 1.96	14.00 + 1.90	18.60 + 6.47	12.80 + 1.35	13.50 + 2.11
1	14.70 + 2.41	21.00 + 1.58	21.80 + 5.01	21.40 + 3.93	22.00 + 2.69
2	18.17 + 3.25	22.28 + 2.81	22.38 + 4.50	18.40 + 3.96	17.75 + 3.75
3	18.67 + 2.02	22.38 + 1.49	23.40 + 2.16	18.13 + 0.48	21.40 + 2.07
4	13.93 + 1.49	15.63 + 1.75	11.75 + 2.22	17.63 + 1.65	11.26 + 2.59
5	15.70 + 1.48	16.30 + 1.04	15.40 + 1.29	14.70 + 2.02	13.75 + 2.88
6	12.82 + 2.75	16.60 + 1.67	16.30 + 1.04	16.64 + 1.90	18.00 + 1.62
8	13. 83 + 1.26	16.83 + 1.04	13.88 + 3.04	17.75 + 1.85	19.38 + 2.02
9	19.60 + 4.36	25.40 + 2.19	22.48 + 2.70	23.92 + 2.24	21.60 + 2.30
10	21.90 + 3.93	24.90 + 0.96	22.00 + 2.18	23.90 + 1.92	21.70 + 1.96
11	18.40 + 2.56	14.70 + 1.04	20.90 + 2.07	16.50 + 2.24	16.30 + 0.76
OVERALL	**16.58 + 2.90**	**19.09 + 4.18**	**18.99 + 4.03**	**18.34 + 3.50**	**17.88 + 3.78**

Table 7: Overall means (+sd) of erythrocyte indices and differential wbc count (x109/l) of groups of shika brow layers fed plain feed (a) or feed containing: 250g/ton (b), 500g/ton (c), 750g/ton (d) or 1000g/ton (e) of superliv® as feed additive during a study period of 12 weeks.

PARAMETERS	GROUPS				P-value
	A	B	C	D	E
MCV (fl)	96.74+7.99	92.47+12.92	99.35+10.57	93.18+13.54	96.54+13.52 0.692
MCH (pg)	32.77+3.02	32.58+8.22	34.23+5.69	31.79+5.86	32.06+5.42 0.901
MCHC (g/dl)	34.24+2.98	33.97+4.00	34.39+2.91	34.70+2.83	33.60+4.45 0.603
Lymphocyte Count ($x10^9$/l)	13.00+2.53	14.98+3.15	14.13+3.36	14.01+2.69	13.60+3.04 0.309
Heterophil count ($x10^9$/l)	2.70+0.80	3.25+1.11	3.07+1.09	3.01+0.74	2.92+0.68 0.190
Monocyte Count ($x10^9$/l)	0.28+0.13	0.34+0.11	0.29+0.11	0.27+0.09	0.27+0.11 0.574
Eosinophil Count ($x10^9$/l)	0.80+0.24	0.74+0.17	0.73+0.23	0.65+0.12	0.84+0.46 0.575
Basophil Count ($x10^9$/l)	0.32+0.15	0.25+0.14	0.33+0.15	0.33+0.17	0.23+0.10 0.072

consistent with herbs acting on animal systems (Craig, 1999).

In conclusion, the current effort has revealed that Superliv, an Ayurvedic herbal feed additive induces beneficial effects on the haematological status of post peak Shika Brown layers under relatively harsh sahel environment. In commercial setting, this is a positive attribute that may qualify the herbal preparation for deployment as useful health enhancement feed additive in relatively older layers under stressful production environment. Though the mechanisms of the positive haematological effects of Superliv in post peak layers are yet to be elucidated, the herbal supplement may act as a haematenic to enhance erythropoiesis in birds. From observations in this study, 250g/ton inclusion level is optimal for Superliv feed supplementation in post peak layers' diet and is hereby recommended.

Impact

The findings of the current study show that Superliv, a proprietary herbal feed additive is a veritable production promoter compound in laying hens, capable of enhancing the blood picture of aging layers with consequent gains through increased productivity and profits. Superliv use as feed supplement in layers is relatively cheaper and safer to the laying hens and consumers of products there from since it is entirely a natural agent of plant origin.

References

Agarwal R, Diswanay S and PATKI P, 1999. Immunomodulatory activities of Withania somnifera extracts in experimental immune inflammation. *Journal of Ethnopharmacology,* 67: 27-35

Asif S M and Wahid S A. 2003. Effects of Fagonia cretica L. constituents on various haematological parameters in rabbits. *Journal of Ethnopharmacology,* 85(2-3): 195-200.

Atteh J O. 2002. Principles And Practice Of Livestock Feed Manufacturing. 1st Edition, Ilorin Nigeria, ADLEK PRINTERS.

Bates J. 1997. Epidemiology of vancomycin –resistant enterococci in the community and relevance of farm animals to human infections. Journal of Hospital Infections, 37: 89-101.

Bolann B J, Rahil-Khazen R, Henriksen H, Isrenn R and Ulvik R J. 2007. Evaluation Methods for trace-element determination with emphasis on their usability in the clinical routine laboratory. *Scandinavian Journal of Clinical and Laboratory Investigation,* 67(4): 353-366.

Brain K R and Turner T D. 1975. The Practical Evaluation of Phytopharmaceuticals. Wright-Science technical, Bristol Britain.

BSAS (2009). Alternatives to antibiotic feed additives for pigs. Issue Papers visited on 31st August, 2009, from http:// www. bsas. org. .

Butaye P, Devriese L A and Haesebrouk F. 1999. Glycopeptide resistance in Enterococcus faecuim strians from animals and humans. *Review of Medical Microbiology,* 10: 235-243.

Craig W J. 1999. Health promoting properties of common herbs. *American Journal of Clinical Nutrition,* 70 (Suppl): 491S-499S.

Clegg M S, Keen C L, Lonnerdal B, Hurley L S. 1981. Influence of Ashing Techniques on the analysis of trace elements in Animal Tissue:1. Wet Ashing. *Biological Trace Element Research* 3: 107-115.

Culer I, 1982. Methodology for the analysis of vegetable drugs. Practical Manual on the Industrial Utilization of medicinal and Aromatic Plants Center Building, Romania pp. 67-81.

FAO/WHO, 2001. Risk Profile on antimicrobial – resistant Bacteria in food. Agenda item 12; Joint FAO/WHO Food Standards Programme Codex Committee on Food Hygiene, 3rd-4th session Bangkok, Thailand.

Francis G, Kerem Z, Makkar H P S, Becker K. 2002. The biological action of saponins in animal systems: A review. *British Journal of Nutrition,* 88: 587-605.

Feighner S D, Dashkevicz M P. 1987. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency and bacterial cholyltaurine hydrolase activity. *Applied Environmental Microbiology*, 53: 331-336

GRAPH PAD INSTAT. 2000. Version 3.05, 32 bit for Win 95/NT, Graph Pad Software, Inc.

Hewitt S S. 1984. *Manual of Veterinary Haematology*. 3rd Edition.

Holmberg S D, Wells J G and Cohen M L. 1984. "Animal to man Transmission of Antimicrobial – Resistant Salmonella; Investigations of U.S. Outbreaks, 1971-1983, Science, 225: 833-835.

Jain N C, 1993. Essentials of Veterinary Haematology. *1st Edition, Philadelphia, Lea and Ferbiger*, USA.

Jibike G I, Arowolo R O A and Oladele O O. 2011. The Effects of Superliv® Supplementation in Feed on Some Production Performance Indeces of Post Peak Shika Brown layers. *Sahel Journal of Veterinary Science,* 10(1): 11-18.

Jukes T H and Williams W L. 1953. Nutritional effects of antibiotics. *Pharmacological Review*, 5: 381- 420.

Lien T F, Horng Y M and Wu C P, 2007. Feasibility of replacing antibiotic feed promoters with the Chinese traditional herbal medicine Bazhen in weaned piglets. *Livestock Production Science*, 107: 92-102.

Mathews K H Jr, 2001. Antimicrobial Drug use and Veterinary Costs in U.S. Livestock Production. USDA. *Agricultural Information bulletin* No. 766 @ww. ers. usda. gov

Michele A, Antonio D M, Renato C, Antimo D M and Augusto P, 2003. Analysis of carbohydrates and amino acids in vegetable waste waters by ion chromatography. *Phytochemical Analysis,* 14(2): 74-82

Morrison J, Bell J, Chang A M and Larkin P K. 1977. A comparative trial of haematinic supplements in pregnancy. Medical Journal of Australia, 1(14): 482-484.

Namkung H, Li M, Gong J, Yu H, Cottrill M, de Lange C F M. 2004. Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. *Canadian Journal of Animal Science,* 84: 697-704.

Nworgu F C, Ogungbenro S A and Solesi K S. 2007. Performance and some Blood Chemistry Indices of Broiler Chicken Served Fluted Pumpkin (Telfaria occidentalis) Leaves Extract supplement. *American-Eurasian Journal of Agriculture and Environmental Science,* 2 (1): 90-9

Odesanmi S O, Lawal L A and Ojokutu SA, 2010. Haematological Effects of Ethanolic Fruit Extract of Tetrapleura tetraptera in Male Dutch White Rabbits. *Research Journal of Medicinal Plants*, 4: 213-217.

Olufemi B E, Assiak I E, Ayoade G O and Onigemo M A. 2003. Studies on the effects of Amaranthus spinosus leaf extract on the haematology of growing pigs. *African Journal of Biomedical Research,* 6: 149-150.

Oyagbemi A A, Saba A B and Arowolo R O A, 2008. Safety Evaluation of Prolonged Administration of Stresroak® in Grower cockerels. International *Journal of Poultry Science,* 7(6): 574-578.

Sarica S, Ciftci A, Demir E, Kilinc K and Yildirim Y. 2005. Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets. *South African Journal of Animal Science,* 35: 61-72.

Scott T A. 2009. Feed additives. Poultry Hub visited on 10th April, 2009 from www.poultryhub.org/ index.php/Feedadditives .

Shivakumar M C, Pugashetti B K, Mulla J A. 2005. Performance promoting ability of herbal liver stimulants in broilers. Visited on 5th May, 2009 from www. poulvet. com/ poultry/ articles/ feed-additives.

Si W, Gong J, Tsao R, Zhou T, Yu H, Poppe C, Johnson R, Du Z. 2006. Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. *Journal of Applied Microbiology*, 100: 296-305.

Smith-Palmer A, Stewart J and Fyfe L. 1998. Antimicrobial properties of plant essential oils and essences against five important food borne pathogens. Letters in Applied Microbiology, 26: 118-122.

Trease G E and Evans W C. 2002. Pharmacognosy 15th Edn. Saunders .

Wang J P, Yoo J S, Lee J H, Zhou T X, Jang H D, Kim H J and Kim I H. 2009. Effects of phenyllactic acid on production performance, egg quality parameters and blood characteristics in laying hens. *Journal of Applied Poultry Research,* 18: 203-209.

Wei A and Shibamoto T, 2007. Antioxidant activities and volatile constituents of carious essential oils *Journal of Agriculture and Food Chemistry*, 55: 1737-1742.

WHO, 2002. Use of antimicrobials outside human medicine and resultant antimicrobial resistance in humans. *WHO fact sheet* No. 268.

Windisch W, Schedle K, Plitzner C and Kroismayr A. 2008. Use of Phytogenic Products as feed additives for swine and poultry. *Journal of Animal Science*, 86: E140-E148.

LOCAL POULTRY FARMERS' MEDIA USE, ACCESS AND UNDERSTANDING OF HIGHLY PATHOGENIC AVIAN INFLUENZA COMMUNICATION MATERIALS IN NIGERIA

Assam A[1], Abdu P A[1], Tabe-Ntui L N[2]
[1]Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria
[2]Department Agricultural Economics and Extension, University of Calabar, Calabar.

Abstract

A two stage household based cluster sampling was conducted in Kaduna State to investigate local poultry farmers' media use, access and understanding to highly pathogenic avian influenza educational materials. Radio and television were appropriate channels for information dissemination to farmers. Television was better at articulating highly pathogenic avian influenza risk perception among farmers. National and State radios and televisions were preferred stations with news and agricultural programs being favourites. Farmers listened to radio in the morning and evening but watch television in the evening. Publication readership was low though farmers look at billboards and posters. Posters pictures were important in attracting farmers' attention. Road junctions, churches, schools and mosques were identified as appropriate locations for placing posters. Radio was an important source of highly pathogenic avian influenza information to farmers though television coverage was poor. High mortality in poultry was what farmers remembered about the highly pathogenic avian influenza radio and television program followed. Farmers' access to highly pathogenic avian influenza educational materials was poor. However, audio-visual and poster were understood better than stickers and bulletins. Highly pathogenic avian influenza message on radio and television should be revised to include disease recognition, the need for reporting sick and dead poultry. Community dialogue system should be established to improve farmers' access to highly pathogenic avian influenza materials.

Key Words: Avian influenza, Bulletins, Communication, Educational materials, Local poultry farmers.

UTILISATION DES MÉDIAS, ACCÈS ET COMPRÉHENSION DES MATÉRIELS DE COMMUNICATION SUR L'INFLUENZA AVIAIRE HAUTEMENT PATHOGÈNE PAR LES AVICULTEURS LOCAUX AU NIGERIA

Résumé

Un sondage en grappes à deux degrés a été mené auprès des ménages dans l'État de Kaduna, dans l'objectif de déterminer, chez les aviculteurs locaux, l'usage des médias, l'accès et la compréhension de matériels d'information sur l'influenza aviaire hautement pathogène. La radio et la télévision étaient les voies appropriées pour la diffusion d'informations aux aviculteurs. La télévision articulait mieux la perception du risque d'influenza aviaire hautement pathogène chez les aviculteurs. Les radios et télévisions nationales et étatiques étaient les canaux idéaux, offrant les informations et les émissions agricoles préférées des aviculteurs. Ceux-ci écoutaient la radio le matin et le soir, mais regardaient la télévision uniquement le soir. Le lectorat des matériels publiés était limité, même si les aviculteurs regardent les panneaux et les affiches. Les images des affiches étaient importantes dans la mesure où elles attiraient l'attention des aviculteurs. Les carrefours, les églises, les écoles et les mosquées ont été identifiés comme des endroits appropriés pour les affiches. La radio était une importante source d'information sur l'influenza aviaire hautement pathogène pour les aviculteurs, mais la couverture de la télévision était faible. L'élément retenu par les aviculteurs des émissions radiophoniques et télévisées suivies sur l'influenza aviaire hautement pathogène était la forte mortalité des volailles. L'accès des aviculteurs aux matériels d'information sur l'influenza aviaire hautement pathogène était médiocre. Cependant, l'audio-visuel et les affiches étaient mieux compris que les autocollants et les bulletins. Les messages sur l'influenza aviaire hautement pathogène passés à la radio et à la télévision devraient être révisés de manière à inclure la reconnaissance des maladies, la nécessité de déclarer les maladies et les mortalités des volailles. De plus, il faudrait mettre en place un système de

Corresponding Author: manassam@yahoo.co.uk

dialogue communautaire pour améliorer l'accès des aviculteurs aux matériels d'information sur l'influenza aviaire hautement pathogène.

Mots-clés: Influenza aviaire, Bulletins, Communication, Matériels d'information, aviculteurs locaux.

Introduction

Local poultry (LP) forming the bulk of poultry population in Nigeria are kept in villages and peri–urban settlements in small number (Abdu *et al.,* 1999). Local poultry are the major sources of poultry meat and eggs (Abdu *et al.,* 1999). Local poultry provides extra income to its owners who are usually poorly educated with meagre resource and occasionally faced with food insecurity (Ahlers *et al.,* 2009).

In Nigeria, LP was estimated at 150 million with about three million in Kaduna state, and they make up to 90 % poultry population (RIM, 2003). Disease especially Newcastle disease (ND) has been identified amongst the constraints limiting LP production (Abdu *et al.,* 1999; Ahlers *et al.,* 2009). The little or non-existence of biosecurity offered by their management system exposes LP to continuous challenge from pathogens and environmental hazards (Abdu *et al.,* 1999). The confirmation of highly pathogenic highly pathogenic avian influenza (HPAI H5N1) in poultry in Nigeria (Joannis *et al.,* 2006) which spread to 97 local government areas (LGA) in 25 States and the Federal Capital Territory (FCT) (AICP, 2009) further increased the constraints on LP production.

Local poultry farmers being stakeholders in the poultry industry need to be involved in measures undertaken to control and contained HPAI in Nigeria. Amongst LP farmers' expected contribution are prompt reporting of HPAI outbreaks and taking measures that would prevent its spread among poultry and reduce human exposure. These can be achieved if LP farmers have the requisite knowledge for recognition of HPAI in poultry and humans, its mode of transmission; preventive measures to reduce spread to poultry and human exposure and proper poultry handling practices (Sowath *et al.,* 2007).

Communication campaigns are aimed at educating the populace through provision of information to develop the requisite knowledge required to reduce human exposure, prevent spread in poultry and ensure reporting. For the educational materials to achieve desired behavioural change, they have to be accessed, understood and accepted by LP farmers, hence the need to identify appropriate communication channels to enhance information dissemination to farmers. This could easily be achieved with an effective extension services which is currently inadequate in most parts of Nigeria (Ejembi *et al.,* 2006). Mass media is presently the major channel of information dissemination even though many LP farmers have limited access (Guẽye, 2009) except the radio (Apantaku *et al.,* 1998; Durosinlorin, 2008).

This study was conducted to investigate LP farmers' media use pattern and assess their access to and understanding of Avian Influenza Control and Pandemic Preparedness and Response Project (AICP) HPAI educational materials.

Materials and Methods

Study Area

Kaduna State is located in North Central part of Nigeria lying between latitude 8° 45" and 11°30" North and longitude 6°11" and 9° East. It has 23 LGA with a population of 6 million people. The average temperature is 34°C with a rainfall of 1,000–1,500 mm. It is bordered by Kastina, Kano, Plateau, Niger, Zamfara, Bauchi, Nassarawa States and FCT. Kaduna state has an estimated poultry population of 2,821092 with about 90% being local poultry (RIM, 2003).

The study was carried out in LGAs where a previous study revealed the presence of low pathogenic highly pathogenic avian influenza (LPAI H5N2) antibodies in LP (Duronsilorin 2008). The LGAs (and villages) were Ikara (Ikara town, Saban Garin Jibis and Gidan Shawai), Kachia (Nasarawa), Lere (Saminaka and Yar kasuwa), Birni Gwari (Unguwan Sarki and Unguwan Shitu), Kagarko (Sabon Garin Kagarko and Tudun Wada) and

Sabon Gari (Dan Gaiya).

Sampling Method

A two stage household based cluster sampling was conducted aimed at 275 respondents – 25 persons in each of 11 villages from six LGA in Kaduna State during the months of August and September, 2009 (Bennett *et al.,* 1991). Within each village, the first household was randomly selected with subsequent household selected by proximity until 25 respondents were enrolled.

Approval was obtained from the community leader to undertake the survey and all respondents provided verbal consent. Information on demographic, access to and understanding of some HPAI stickers, posters, bulletin and a video clip prepared by Nigeria's Highly pathogenic avian influenza Control and Human Pandemic Preparedness and Response Project (AICP); media listened to, read or watched and their preferences; their understanding and influence of HPAI information on their poultry handling practices were collected through an interview using a standardized structured questionnaire. Additional probe questions were also asked, where necessary, to shed more light on some issues raised during the administration of questionnaire.

Five posters, two stickers, a bulletin and an AICP video advert were used in assessing LP farmers' access and understanding of AICP educational materials. The video advert was played to farmers using a laptop. It discussed HPAI transmission, the need to avoid contact with poultry and seek medical attention in case of flu-like illness after contact with poultry.

An interpreter assisted in interpreting questions from English to Hausa for participants who did not understand English. Data was analyzed by descriptive statistics using SPSS version 17.0 (SPSS Inc. Chicago, IL, USA).

Results

One hundred and seventy-three respondents were interviewed and questionnaires filled with 52.6 % males and 62 % aged between 25–44 years. Two–third of the respondents had secondary education with 36.4 % housewives and 24.9 % farmers being the main occupation groups.

Fifty–two per cent of farmers listened to national radio with 42.2% listening to state radio and 2.9% do not listen to radio. Amongst those who listened to radio, 67.5% listened to news and 15.4 % listened to agricultural program. There was association between the radio station farmers listened to and the program they listened with 71.2% of State radio listeners preferring news and 12.3% agricultural program ($X2 = 66.42$; $p= 0.01$). Males either listened to the radio alone (52.9%) or with friends (73.2%) while females listen with family (58.5%) or family and friends (60%; $X2 = 12.10$, $p = 0.02$). Forty two per cent of farmers listened to radio in the evenings with 34.3 % preferring mornings and 12.4% listened both in the mornings and evenings. However, the most convenient time to listen to the radio was evenings for 56.2% of the farmers. Thirty-five per cent of LP farmers who listened to State (35.6%) and 46.7 % National radio stations listeners favoured listening in the evenings ($X2 = 69.65$, $p = 0.00$). Over 40 % of farmers listened alone with 31.4% listened with family and 24.3% with friends. The study revealed that farmers who listened to radio in the morning listen alone (53.4%) while those who listened in the afternoon (41.2%) listened with family members and evening listeners (36.6%) usually listened with friends ($X2 = 48.23$, $p = 0.00$). State radio listeners (46.6%) listened alone compared to national station listeners who listened with either family (34.4%) or friends (26.7%) though generally, farmers either listened with family (31.4%) or friends (24.3%) or both (4.2%; $X2 = 53.53$, $p = 0.00$). Farmers who listened to news listened in the morning and alone (45.6%) although those who listened to agricultural programs listened in the evenings with family members (53.8%; $X2 = 75.21$, $p=0.00$).

Forty-one per cent of LP farmers do no read any publication with 50.9% reading newspapers, 6.4% magazines and 1.7% reading bulletins. However, 28.3% read the publications often with only 19.7% who read very often though less than a quarter of the farmers get their publications often. Farmers read publications

either in English (49.5%) or Hausa (42.7%). Among farmers who read newspapers, 47.7% get their papers often while 63.6% of magazine readers get their magazines very often (X2 = 178.98, p= 0.00) though 45.5% of newspapers were read in either English or Hausa while 90.9% of magazines were in English. However, all the bulletins read were in Hausa (X2 = 13.26, p= 0.04). News (56.9%) and agriculture (37.3%) programs were the items enjoyed by farmers who read publication. News was the favourite section of 64.8 % of newspaper readers though 90.9 % who read magazines and bulletins preferred agricultural section (66.7%; X2 = 20.23; p = 0.03).

Over three–quarter of farmers watch television (TV) with 43.4% preferring National TV and 38.7% State TV. However, 79.2% favoured news with only 18.9 % preferring agricultural programs. Seventy one per cent of the farmers watch TV in the evenings. TV was watched with either family members (42.8%) or friends (23.3%) though 34% watched alone. Farmers watching State (68.2%) and national (81.3%) TV do so in the evenings (X2 = 34.25, p=0.00) with 37.3 % of National and 42.4 % State (42.4%) TV viewers watching with family unlike private TV viewers who watch alone (55.6%; X2= 22.79, p = 0.00). News (40.9%) was the favourite program of farmers in both State (24.2%) and National (17.3%; X2 = 210.07, p = 0.00) TV with the most convenient time to watch their favourite program being evening for State (66.7%) and National (80.0%; X2 = 56.33, p = 0.00) TV. There was association (p = 0.00) between the time LP farmers watch TV and the time convenient to them with the morning being the most convenient for 75% morning viewers and 67.6% for evening viewers.

State radio listeners (54.8%) do not read any publication and 60% of National radio listeners read newspapers (X2 = 29.1, p= 0.02). However, 40 % of farmers who do not listen to radio watch either State or National TV (X2 = 46.94, p = 0.01).

When respondents were asked if they look at posters or billboards, Eighty-eight per cent of LP farmers look at posters and billboards. Farmers usually see the posters or billboards by the road (72.3%), school (12%) or church (11.2%). However, 93.5% of farmers were attracted to these messages because of their pictures. Farmers recommended road junctions (78.5%), churches (6.4%) and mosques (14.0%) as the most appropriate site for placement of posters and billboards.

A relationship exists between farmers who look at posters and placement site, with posters placed at road junctions (73.2%; X2 = 83.62, p=0.00). Farmers who do not look at billboards/posters recommend the mosque as ideal site for placement (47.4%; X2 = 34.67, p = 0.00). Picture in posters/ billboards attracts farmers to them (94.2%; X 2 = 76.5, p=0.00). Secondary educated farmers (64. 6%; X 2 = 13.49, p = 0.04) were attracted to posters/ billboards by their pictures. However, more female (51%) look on billboards than males (X 2 = 6.81, p=0.01).

Among the 71.1% of farmers who heard about HPAI on radio, 66.9% heard from National radio. Generally, 63.4% of farmers heard HPAI from news with 27.6% from agricultural programs. However, most farmers who heard HPAI on State radio heard in the news (80.5%) while those who heard on the National radio heard from an agricultural program (32.9%; X 2 = 12.31, p = 0.02). Farmers who listened to State radio (56.4%) heard about HPAI on State radio while 84.8% of National radio listeners heard about HPAI on National radio (X2 = 24.54, p=0.00). Over 71 % of farmers who heard HPAI on National radio had secondary education though only 2.4% of Islamic educated farmers heard about HPAI on National radio with 7.3% having heard on state radio (X 2 = 6.36, p = 0.04). Farmers who heard HPAI on radio listened to the program with family (90.6%), friends (61%) or family and friends (80%) though 64.7% listened alone (X 2 = 14.47, p=0.01). Seventy per cent of those who listen to HPAI program alone and with family (58.3%) heard about HPAI in the news while those who listen with friends (48%) heard in agricultural program (X 2 = 28.03, p=0.03).

Fifty – two per cent of farmers who read publications had not read about HPAI in any publication though amongst those who have read about HPAI, 28% read in Daily Trust newspaper (Fig. 1). The language of publication on HPAI were English for Daily Trust (29.6%),

Hausa for GTK (56.3%) and English and Hausa for New Nigerian (85.7%; X2 = 50.10, p = 0.00).

Ninety-eight per cent of farmers had neither seen nor read the AICP bulletin on HPAI.

About a third of respondents who watch TV had watched a TV program on which HPAI was mentioned though 65.2% farmers watched the program on National TV with less than a third who watched on state TV (p =0.00). However, 94.4% of respondents who watch National TV watch HPAI program on National TV with over three-quarter of State TV viewers having watched the program on State TV (X2 = 118.14, p = 0.00). When showed an AICP TV advert on HPAI, 93.6% of farmers reported they have never watched or listened to the advert.

When farmers were presented with five AICP sponsored posters, 75.7% of farmers had not seen any of the posters with only 2.3% who have seen all 5 posters. Likewise, 91.9 % of farmers had never seen any of the two AICP stickers presented though only 1.2% of the farmers had seen both stickers.

Over 78.6% of farmers who recommended road junction as placement site for posters had not seen any of the AICP posters (X2 = 210.53, p= 0.00). Over a third of those who suggested the mosque as an ideal place for posters placement were between 25 – 34 years old (X2 = 35.82, p = 0.01).

When asked what farmers remembered from HPAI program listened to from radio, responses include it causes high mortality (19.1%), or it being a serious disease (13.9%) amongst others (Fig. 2). National radio listeners, who heard about HPAI on National radio, remembered that HPAI causes high mortality (26.8%) in poultry and the need to report sick or dead poultry (1.2%). State radio listeners remembered to protect self from HPAI (19.5%) and that HPAI is a serious disease (Fig. 3).

Morning listeners (17.4%) remembered the need to protect self compared to evening listeners (6.7%) though only 2.2% of evening listeners remembered cooking poultry properly against 6.5% of morning listeners (X2 = 86.52, p = 0.00). A quarter of those who listened to HPAI program with family and friends could remember only the need to wash hands with soap and water (X2 = 95.18, p = 0.00). However, those who heard about HPAI in the news could only remember that it is a serious disease (30.8%) while those who heard in an agricultural program (26.5%) remembered that it causes high mortality in poultry (X2 = 102.52, p=0.00).

Over three quarter of farmers who have never read the AICP bulletin reported that the message would not affect their behaviour (76.6%) though all those who have read the bulletin prior to the study revealed that the message would change their behaviour (X2 = 45.79, p=0.00). High mortality in poultry was what 47.8 % of farmers remembered of the HPAI program watched on TV. State TV viewers remembered the need to protect self from HPAI (13.6%; X2 = 91.97, p= 0.00).

Upon watching the AICP TV advert, 56.6% of the farmers reported understanding the advert and that it would positively affect their behaviour towards handling poultry.

Of the farmers that had not seen any of the 5 AICP posters presented, 67.1% understood the messages of all the posters when showed. Farmers who did not understand the posters, indicated posters P1 (27.1%) and P3 (11.9%) as the posters they did not understand with 61% of farmers not understanding any of the posters. The posters would affect 61.8% farmers' attitude towards poultry. However, two–third of farmers revealed that they did not understand the messages on the stickers and the messages would not affect their poultry handling practices.

Over seventy–one per cent of farmers who look at billboards and posters understood the message of all the AICP posters (X2 = 10.2%; p = 0.00) though 35.3 % reported the message would not affect their attitude toward poultry handling (X2 = 4.79, p = 0.03). Fifty–five per cent of farmers who look at billboards and posters do not understand the message on the stickers (X2 = 4.79, p = 0.03). Sixty – eight per cent of respondents who suggested road junction as the best place for posters/billboards understood the posters' messages though all farmers who recommended the mosque for posters placement did not understand the poster message (X2 = 12.13, p = 0.02). Eighty-

six per cent of farmers who view posters or billboards because of the site of placement understood the messages of stickers although 56.3% of farmers attracted by the pictures of posters did not understand stickers messages ($X2= 7.94$, $p=0.04$) with 62.5% reporting the stickers messages would affect their poultry handling practices.

Discussion

The study revealed that despite the constraint of non-availability of or erratic power supply and expenses involved in maintaining radio and television, they are preferred sources of information to LP farmers (Apantaku *et al.,* 1998; Gueyè, 2009; Igwe *et al.,* 2008; Durosinlorin, 2008) and are practicable sources of agricultural information. The finding is contrary to previous reports of low TV viewership and inability of LP farmers to watch TV because they cannot afford TV (Okwu *et al.,* 2006; Gueyè, 2009).

News were the favourite programs of radio listeners and TV viewers, implying that information for LP farmers can be disseminated through news (Adekunle *et al.,* 2004). However, State and National stations were favourite stations contrary to a study by Adekunle *et al.,* (2004), where Ago-Are and Tede communities' arable farmers' favourite TV and radio stations were State and private stations. This difference might be as a result of proficiency of these arable farmers in English Language as state and private stations broadcast programs in the local language.

Though the widest radio listenership was obtained in the mornings and evenings, the afternoon radio listeners were usually women and listened with family members mainly children when preparing the evening meal while their husbands are at work. These women are equally important in HPAI control as they are usually the careers of local poultry (Abdu *et al.,* 1999; Ahlers *et al.,* 2009).

Readership of publications among LP farmers was poor probably because they do not address issues of interest to LP farmers or farmers cannot afford to buy publications due to high cost. Contrary to previous reports that attributed low publication readership to illiteracy of farmers (Okwu *et al.,* 2006), this study revealed most LP farmers had secondary education. The poor readership of publications reported in this study is contrary to a study carried among commercial poultry farmers in Ibadan, Nigeria (Aderinto and Adisa, 2006) which reported higher readership of newspapers due to high literacy level of the farmers. Though magazines are more expensive, they are usually read by the educated LP farmers. However, bulletins are usually published in Hausa and given out free as they carry information to farmers.

Billboards and posters placed in schools, churches and road junctions were suggested as appropriate media channels for LP farmers contrary to the report by Okwu *et al.,* (2006). The colourful pictures of posters and billboards attract LP farmers who are usually poorly educated as they proffer visualisation making the posters and billboards self explanatory (Mgbada, 2006; Ahlers *et al.,* 2009) though contrary to reports by Ejembi et al. (2006) that indicated difficulties in interpreting pictures by farmers in Makurdi LGA in Benue State, Nigeria. This study revealed that farmers would not understand a single poster addressing many themes or when poster picture does not clearly show what is expected from the audience.

Secondary school graduates listen to National radio because they understands the English language hence read publications unlike Islamic educated LP farmers who would prefer State radio which broadcast in English and 'Hausa' and have more Islamic oriented programs. The study revealed that TV could be an alternative channel of information distribution to some non-radio listeners.

Similar to previous report, radio was a key medium of informing LP farmers about HPAI (Durosinlorin, 2008) unlike TV which was poorly utilized for HPAI coverage contrary to reports by Aderinto and Adisa (2006). Poor TV coverage resulted in the poor recognition of HPAI among LP farmers as the audio-visual advantage involving more human senses of sight, hearing and feeling in analysing message ensuring higher chances of behavioural change was not utilized by LP farmers (Adedoyin and

Adebayo, 2005).

State radio and TV stations performance on HPAI information dissemination was poor which might have a negative impact on HPAI education of LP farmers who resides in rural communities where State radio and TV reception are better compared to National stations. State radio and TV programs are usually broadcast in the local language which farmers understand better and these programs are design considering farmers' socio-cultural and economic background. The poor coverage by State media might be as a result of inadequate state media personnel knowledgeable on HPAI; inability of media management to involve state veterinarians in HPAI programs or poor perception of media management on HPAI risk perception and how it affects the rural communities.

The study revealed that the appropriate time to broadcast HPAI information targeting LP farmers should be in the mornings and evenings through the radio (Anigwe, 1990; Adekunle *et al.,* 2004) and in the evenings through the TV (Nwachukwu and Akinbode, 1989; Adekunle *et al.,* 2004). Though morning radio listeners listen alone, they could discuss the information with friends and colleagues while performing their daily chores. However, evening radio listeners (Adekunle *et al.,* 2004) and TV viewers listen or watch HPAI programs with family and/or friends enabling issues raised in the program to be discussed among farmers which might improve the understanding of the issues for those who did not understand.

The LP farmers need for agricultural information was reflected in their viewership or listenership of agricultural programs. These farmers are important as entry points for introduction of HPAI knowledge in their communities as they are considered more receptive to innovations (Adekoya and Tologbonse, 2005).

However, TV would complement radio and articulate the risk perception of LP farmers on HPAI better while improving the chances of behavioural change of farmers' risky poultry handling practices contrary to reports by Okwu *et al.* (2006) that TV would be counter-productive as a means of disseminating agricultural information to rural communities.

Radio listeners and TV viewers could recall mainly high mortality in poultry and that HPAI is a serious disease from HPAI program watched. This was as result of information source been the news and explains the lack of behavioural changes as LP farmers continue to practice HPAI risky behaviours such as eating and selling of sick or dead poultry, living closely with poultry, use of poultry faeces for manure and inability to report poultry death (Durosinlorin, 2008; Fasina *et al.,* 2009). There is the need to re-evaluate HPAI message being broadcasted (Okwu *et al.,* 2006) in the radio and TV to include disease recognition, the need to report sick and dead poultry, where to report disease outbreak and avoidance of HPAI risky behaviours.

Though state radio reported the need to protect self, the only protective measures LP farmers could remember were wash hands with soap and water and cook poultry properly which might be because these practices are similar to their cultural practice hence the ease of identifying with the practices. Discussion with some LP farmers revealed that the commercial poultry are source of HPAI rather than the indigenous poultry hence the need to protect self from commercial and not indigenous poultry further highlight the gap in HPAI message packaging by the media.

The study revealed that placing HPAI information in the news section of a newspaper or agricultural section of magazines and bulletin would ensure wide readership among LP farmers. The inability of more than half of the publication readers not to read about HPAI in their publication might be that HPAI has not been mentioned in the readers' favourite section of the publication

The relationship of what farmers remembered from HPAI program with the station and program that information on HPAI was heard; time of listening to the program and person they listen with is similar to previous study (Okwu *et al.,* 2006). This is because the time of listening to the program determines who they listen with and possibility of ensuring discussion clarifying the issue to those who did not understand the message. The discussion generated by the HPAI program would also ensure long term memory retention of issues

discussed (Adekoya and Tologbonse, 2005) which might create a favourable condition for listeners and viewers to attempt a trial if the person who understands the issues discussed in the program is respected and trusted within the community (Adekoya and Tologbonse, 2005).

The low distribution of the AICP TV advert among LP farmers might be due to the low coverage of HPAI by state TV possibly because of little or non involvement of state TV in ACIP sponsored HPAI programs. The ability of the advert to bring about behavioural change is because more human senses are involved in evaluation of the message as seeing the pictures and hearing message ensure better understanding and a sense of participation (Lawal-Adebowale and Adebayo, 2006; Adebayo *et al.,* 2008). There is the need for AICP to involve the State TV and radio in dissemination of HPAI information through sponsorship of HPAI programs on State radio and TV.

The poor circulation of AICP bulletins, posters and stickers among LP farmers is because the agricultural extension services is apparently non-existing or the LGAs AICP information desk officers are not engaging the extension services in HPAI control. It might also be that these educational materials are not sent to the rural communities but left in AICP offices at federal or state AICP secretariats. In some instances, stickers and posters were given only to individuals favoured by the LGAs information desk officers as some LGAs AICP animal health desk officers had not seen most of the materials. The poor circulation of HPAI educational materials could be ameliorated with the establishment of a functional community dialogue system (CDS) as recommended in the Nigeria's Highly Pathogenic Avian Influenza Preparedness Plan (AICP, 2007).

The inability of the bulletins to bring about attitudinal change might be because LP farmers can not perceive the risk of HPAI from the bulletin as a result of the need to reflect on message in bulletin which takes a longer time as confirmed by the study. The study indicates that publications are not good channel for HPAI information dissemination to LP farmers.

Placement of billboards/posters in mosques would improve Islamic educated LP farmers access to HPAI information as their radio listenership and publication readership were poor coupled with their important role in the LP production chain (Assam, Unpublished). Moslem youths could serve as initiators of HPAI advocacy campaign among the Moslem communities who are less informed on HPAI control.

These posters and billboards can adequately convey the risk involved in HPAI risky practices resulting in avoidance of these practices. The AICP posters if understood could induce desired behavioural change among LP farmers for the aforementioned reasons.

The absence of pictures makes it difficult for farmers to understand stickers' messages and effect appropriate changes on their poultry handling practice that would assist in HPAI control.

The results of this study may be broadly generalized to the southern part of the country despite the socio-cultural differences. However, it is also applicable to other states in Northern Nigeria and neighbouring countries like Niger Republic, Cameroon and Chad sharing cultural similarities.

In conclusion, radio, television, billboards and posters are viable channels of information dissemination to LP farmers. However, LP farmers' access to HPAI educational materials was very poor though television and posters' messages were better understood than bulletins and stickers' messages.

Conclusions

Radio and television are viable channel for HPAI information dissemination to LP farmers unlike publications readership which was low. However, television is better at articulating HPAI risk perception of LP farmers though State owned radio and TV coverage of recent HPAI outbreak was inadequate. Farmers listen to radio in the morning alone although evening radio listeners or TV viewers listen or watch with family, friends or family and friends. News and agricultural programs were favourite programs for LP farmers who read publications or watched television or listened to radio. Billboards and posters had high viewership and were easily understood by LP farmers

though pictures in posters and billboards were important at attracting the attention of LP farmers.

High mortality in poultry was what farmers remembered of the HPAI program listened on radio or watched on television. Distribution of AICP educational materials among LP farmers was low. Audio-visual and posters message were better understood by LP farmers unlike bulletin and stickers which were not clear.

Recommendations

Highly pathogenic avian influenza information targeting LP farmers should be channelled through radio, television or posters and billboards. State radio and television should increase HPAI programs in their schedules and ensure human resource development in HPAI information broadcasting. Nevertheless, radio and television HPAI message should be revised to include disease recognition, need for LP farmers to report and where to report sick or dead poultry and the need to avoid HPAI risky behaviours. Muslim youths should be engaged to HPAI advocacy within Muslim communities.

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References

Abdu P A, George B D J and Sa'idu S N A. 1999. The production management and health of village chickens in Kaduna State, Nigeria. Book of Proceedings: 26th Annual Nigerian Society of *Animal Production Conference, 21 – 25 March,* Ilorin.Pp.474-475.

Adedoyin F S and Adebayo K. 2005. Communication and advocacy strategies in extension. In: Adedoyin, S. Fola (ed.): *Agricultural Extension in Nigeria. Agricultural Extension Society of Nigeria*, Pp. 21 – 27.

Adekoya A E and Tologbonse E B, 2005. Adoption and diffusion of innovations: In: Adedoyin, S. Fola (ed.): Agricultural Extension in Nigeria. *Agricultural Extension Society of Nigeria (AESON)* Pp.28 – 37.

Adekunle A A, Olowu T A, Ogunyinka O and Olubodun B. 2004. Farmers' media usage pattern and adoption of agricultural technologies in Ago – Are and Tede communities. Information and Communication Support for Agricultural Growth in Nigeria (ICS-Nigeria) *Project document.* Pp. 7-14.

Aderinto A and Adisa A T. 2006. Poultry farmers' knowledge of management practices involved in the prevention and control of bird flu in Ibadan/Ibarapa agricultural zone, Oyo State. Proceedings, *Eleventh Annual Conference AESON, 3 rd – 6 th April, 2006.* Pp. 228 – 234

AICP. (2009). Summary of depopulation of birds and compensation payments made. Bird Flu Watch, Vol. 1 No. 2, Pp. 12.

Avian Influenza Control Programme (AICP), 2007. The Integrated National Avian Influenza and Pandemic Response Plan.

Ahlers C, Alders R, Bagnol B, Cambaza A, Harun M, Mgomezulu R, Msami H, Pym B, Wegener P, Wethli E and Young M. 2009. Improving local chicken production: A manual for field workers and trainers. *Australian Centre for International Agricultural Research Monograph* No. 139. Pp:123.

Anigwe O E. 1990. Farm-radio listenership and agricultural knowledge: a case study of farmers in Akinyele Local Government Area of Oyo State, Nigeria. Unpublished MSc. Thesis, *Department of Agricultural Extension,* University of Ibadan, Ibadan.

Apantaku S O, Apantaku F S and Oyesola O B. 1998. Rural poultry farmers' use of electronic mass media and level of productivity in Egbeda, Nigeria. Proceedings of Silver Anniversary Conferences of the Nigerian Society for Animal Production (NSAP) and the Inaugural Conferences of the West African Society for Animal Production (WASAP), Abeokuta, Nigeria, pp. 526 – 527.

Bennett S, Woods T, Liyanage W and Smith D. 1991. A simplified general method for cluster-sample surveys of health in developing countries. *World Health Statistics Quarterly,* 44:98–106.

Durosinlorin A. 2008. Epidemiological study of H5N2 antibodies in rural chicken and knowledge and awareness level towards HPAI (H5N1) among

rural poultry farmers in Kaduna State, Nigeria. *MSc. Thesis, Ahmadu Bello University,* Zaria – Nigeria. 96 pp

Ejembi S A, Ejembi E P and Okwoche V A. 2006. Problems of effective adoption decision among farmers in Markurdi, Benue State: The relevance of choice of communication methods. *Proceedings, 11 th Annual Conference, AESON, 3rd – 6th April, 2006*. Pp. 235– 241.

Fasina O F, Bisschop S P R, Ibironke A A and Meseko C A. 2009. Highly pathogenic avian influenza risk perception among poultry workers, Nigeria. *Emerging Infectious Diseases,* 15 (4): 616 – 617.

Gueyè E F. (2009). The role of networks in information dissemination to local poultry farmers. *World Poultry Science Journal*, 65: 115 – 124.

Igwe K C, Nwadinigwe C O and Iheke O R. 2008. Awareness of the incidence and sources of prevention of bird flu disease among poultry farmers in Imo State, Nigeria. *Proceedings, 13th Annual Conference, AESON, 8th – 11th April, 2008*. Pp. 76 - 79

Joannis T, Lombin L H, De Benedicts P, Cattoli G and Capua I. (2006). Confirmation of H5N1 highly pathogenic avian influenza in Africa. *Veterinary Records*, 158: 309 – 310.

Lawal-Adebowale O A and Adebayo K. (2006). Information and communication technologies: Pathways to effecting feedback in mass extension method of message delivery. *Proceedings, Eleventh Annual Conference AESON, 3rd – 6th April, 2006*. Pp. 184 – 190.

Mgbada J U. (2006). Effectiveness of sources of information to women farmers from accessible and non-accessible localities in Enugu State. *Proceedings, Eleventh Annual Conference AESON, 3rd – 6th April, 2006.* Pp. 72 – 81.

Nwachuku I and Akinbode I A. (1989). The use of television in disseminating agricultural technology: a case study of selected communities in Oyo State, *Nigeria, Rural Development* in Nigeria, 3 (2):111-115.

Okwu P, Obinne C P O and Umeh J C. (2006). A study of mass usage in agricultural extension in Benue State of Nigeria. *Proceedings, Eleventh Annual Conference, AESON, 3rd – 6th April, 2006*. Pp. 9 – 17.

RIM Report. (1993). Nigerian Livestock Reserve Resource Inventory & Management Report, Vol. 1-4. *Federal Department of Livestock and Pest Control Services*.

Sowath L, Van Kerkhove D M, Holl D, Froehlich Y and Vong S. (2007). Interaction between humans and poultry, rural Cambodia. *Emerging Infectious Diseases*, 13 (1):130 – 132.

SHORT COMMUNICATION

A TYPICAL ACTINOBACILLOSIS IN AN ADULT FRIESIAN COW

Thaiyah A G*, Aleri J W, Abuom T O and Mulei C M.
Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi
P.O. BOX 29053 – 00625 Kangemi, Kenya

Actinobacillosis due to Actinobacillus lignieresi, has been reported in domestic animals including cattle, sheep, goats, buffalo, horses and dogs (Carmalt *et al.,* 1999; Kennerman *et al.,* 2006; Muhammad *et al.,* 2006; Brown *et al.,* 2007). The causative agent is a gram-negative aerobic rod and a normal inhabitant of the gastrointestinal tract of ruminants (Radostits *et al.,* 2007; Smith, 2009). The most frequent clinical presentation is granulomatous or pyogranulomatous lesion of the tongue or subcutaneous tissues in the head and neck region. A typical manifestation of the disease have been reported affecting other body tissues / organs usually associated from lacerations, dehorning, nose-rings, intravenous injections and lymphadenitis (Aslani *et al.,* 1995; Holzhauer and Roumen, 2002). Outbreaks of the disease have also been reported (Campbell et al., 1975; Nakazawa and Azuma 1977).

This paper outlines an atypical case of actinobacillosis of soft tissue swelling of the head in a cow without any exudation from the lesion, which is a challenge to confirming the etiological agent and treatment.

An adult Friesian cow had been referred to the Large Animal Clinic, University of Nairobi, Kenya with a history of a longstanding bilateral swelling of the face over a period of two months. The patient had been treated by a private veterinarian with unknown dosages of antibiotics without any success.

On clinical examination the patient had a bilateral swelling of the face (Figure 1) resembling a hippopotamus. The swelling had extended from the ramus of the mandible to the muzzle and upper jaw region. On palpation, the swelling was firm and non-painful. The patient also presented with hypersalivation, increased nasal discharge and slightly swollen sub-mandibular lymph nodes. All vital parameters were within the normal ranges and she had a pregnancy aged 4 months.

Treatment regime involved administration of 300 milliliters of 10% Sodium iodide through the jugular vein three weeks apart, followed with seven days injection of 4g procaine penicillin and dihydrostreptomycin intramuscularly.

The lesion regressed progressively for up to a period of 3 months. Signs of iodism appeared during the course of treatment (Figure 2) and regressed once the treatment was discontinued.

Actinobacillosis in cattle is usually represented by the classical "wooden tongue' syndrome. However, other forms have also been described including the unusual form presenting with wart-like lesions on the dorsum of the tongue (Taghipour Bazargani, *et al.,* 2010). In this case, the soft tissue swelling presenting as a "hippo-head" was firm, non-painful with no discharge. A similar case had been reported previously (Milne *et al.,* 2001). However, in the case reported by Milne *et al.,* 2001 the facial swelling was accompanied by granulomatous swellings with mucoid discharge unlike in the present case where the swelling was smooth and without discharge. The prolonged nature of the case could not allow for an aspirate to be sampled as it has been shown that it's difficult to grow the causal agent after a prolonged antibiotic treatment (Fubini and Campbell, 1983). However, a biopsy could be taken but was not attempted due to the severity of the lesion and the requirement for deep skin incisions when taking biopsies (Milne *et al.,* 2001).

Many treatments have been tried for actinobacillosis including surgical debulking followed by postoperative therapy with intramuscular penicillin/streptomycin and intravenous sodium iodide (Arora *et al.,* 1980), streptomycin/or dihydrostreptomycin (Prescott

Keywords: "Hippo-head" presentation, Sodium iodide, actinobacillosis

*Corresponding Author: andrew.thaiyah@gmail.com

and Baggot, 1993) and other antimicrobials including tetracycline's and sulphonamides (Prescott and Baggot, 1993, Radostits *et al.,* 2000; Milne *et al.,* 2001). In the present case sodium iodide was the treatment of choice due to the failure of earlier antibiotics therapy and resulted in success. However, it is important to look out for signs of iodism which signifies an end point to treatment with sodium iodide.

It is therefore important for veterinarians who come across such cases of swollen face to recognize this form of actinobacillosis which is different from the classical "wooden tongue" and to institute treatment with Sodium iodide before condemning the animals.

References

Arora B M, Parihar N S and Bhargava A K. (1980) Treatment of a case of cutaneous actinobacillosis in cattle. *Indian Veterinary Journal* 57,771-772

Aslani M R, Khodakaram A and Rezakhani A. (1995). An atypical case of actinobacillosis in a cow. *Journal of American Veterinary Medical Association* 42:485-488.

Brown C C, Baker D C and Barker I K. (2007). Alimentary system In: Maxie MG, Jubb, Kennedy and Palmers Pathology of domestic animals. Volume 2, 5th edn, *Elsevier Saunders*, Pp.20-22.

Campbell S G, Whitlock T R H, Timoney J F and Underwood A M (1975). An unusual epizootic of actinobacillosis in dairy heifers. *Journal of American Veterinary Medical Association* 166:604-606.

Carmalt J L, Baptiste K E and Chirino-Trejo J M. (1999). Actinobacillus lignieresii infection in two horses. *Journal of American Veterinary Medical Association* 215:826-828.

Fubini S L and Campbell S G. (1983) External lumps on sheep and goats. *Veterinary Clinics of North America - Large Animal Practice* 5, 457-476

Holzhauer M and Roumen T P. (2002). Atypical actinobacillosis in a dairy replacement herd. *Veterinary Record* 151:276.

Kennerman E, Salci H and Sevimli A. (2006). Case of systemic actinobacillosis in a dog-A case report. *Bulletin of the Veterinary Institute in Pulawy* 50:613-617.

Milne H O, Barrett D C, mellor D J and O'Neill J, Fitzpatrick J L. (2001). *The Veterinary Record. Short communication March 3*. pp 273-274.

Muhammad G, Saqib M and Athar M. (2006). Fatal actinobacillosis in a dairy Buffalo. *Acta veterinaria* BRNO 75:247-250.

Nakazawa M and Azuma R. (1977). Collective outbreaks of bovine actinobacillosis. *Japanese Journal of eterinary Science* 39:549-557.

Prescott J E and Baggot J D. (1993) Aminoglycosides and aminocyclitols.In Antimicrobial Therapy in Veterinary Medicine. 2nd edn. Ames, *Iowa State University Press*. pp 144-178

Radostits O M, Gay C C, Blood D C and Hinchcliff K W. (2000) Diseases caused by bacteria - IV. *In Veterinary Medicine*. 9th edn. London, W. B. Saunders. pp 909-944

Radostits R M, Gay C C, Hinchcliff K W and Constable P D. (2007). *Veterinay Medicine*. 10th edn, Pp. 1046-1048.

Smith B P. (2009). Large Animal Internal Medicine. 4th edn, C.V. Mosby, Philadelphia, Pp. 782-784.

Taghipour Bazargani Khodakaram T T, Atyabi A and Faghanizadeh N Gh. (2010). An Unusual occurrence of Actinobacillosis in Heifers and Cows in a adairy herd in Tehran Suburb-Iran. Archives of razi Institute, vol 65:2 pp 105-110.

SHORT COMMUNICATION

EFFECTS OF REFINED PETROLEUM PRODUCT (KEROSENE) FLAME AND FUMES ON HAEMATOLOGICAL CHARACTERISTICS OF BROILER CHICKENS

Amakiri A O*, Owen O J and Jack D O
[1]Department of Animal Science Rivers State University of Science and Technology,
P. M. B. 5080, Port Harcourt, Nigeria.

Introduction

Petroleum (crude oil) is a remarkably varied substance both in its use and composition. It is often dark, straw coloured, tar black or sometimes green in outlook. Crude oil is the chief source of hydrocarbons and when fractionally distilled, the various components are often collected over a range of boiling points (Ababio, 1993). The main fractional distillates of petroleum include natural gas, light petroleum (petroleum ether), ligroin (light naphtha), petrol (gasoline), paraffin (kerosene), gas oil, lubricating oil and asphalt (bitumen).

Refined petroleum product (kerosene) is a mixture of hydrocarbons that contains 12-18 carbon atoms per molecule and it boils between 190 - 250°C. It is a fairly volatile liquid which is used as a fuel for lighting lamps (illumination), heating or cooking, fuel for automobiles driving, modern jets and aeroplanes, burning bush, grasses and wood (incineration) (Murray, 1972; Jumoke, 1999). Kerosene is also a good solvent for grease and paints. It is also used as an insect repellant because of its odour. Developing countries with epileptic electricity supply use kerosene in lanterns and stoves for heating and brooding chicks and other livestock. When kerosene burns, it produces a flame which could be blue, luminous flame or yellow sooty flame, depending on the type of burner used. Fumes from sooty kerosene flame are laden with Volatile Organic Carbon (VOC) and Suspended Particulate Matter (SPM), which irritate the respiratory tract when inhaled either by man or livestock.

Clinical evaluation of blood parameters is very important to monitor and evaluate disease prognosis (Igene and Arijeniwa, 2004; Eniolorunda *et al.,* 2005, Owen *et al.,* 2008). Kaneko (1989) concluded in his study that it is often very difficult to assess the current health status of the animal without detailed examination of the blood. Haematological parameters have been evaluated in many different species of animals by researchers. These parameters are known to change with such factors as environment, nutrition, health condition, age, sex, season, strain or breed and physiological state.

White (1975) reported that fluoride administered to rabbits influenced the reduction in the number of blood lymphocytes, erythrocytes, eosinophils, as well as the structure and function of lymphoid tissue. Saita (1974) demonstrated that benzene on the other hand, a product of crude oil induces leukemia, erythropenia, neutrophilia, lymphocytosis and alteration in platelet morphology in animals.

Ngodigha *et al.,* (1999) also reported that in their work on goats, lymphocytes decreased as the crude oil contamination increased. The crude oil has been found to change the blood chemistry and cause antibody depression, lymphatic involution among other effects and the release of ACTH and glucocoticoids in stressed animals. In another work conducted by Wekhe et al, (2001), rabbits were treated with crude oil contaminated feed at graded levels of 0.01%, 0.02% and 0.03%, it was reported that a significant high ($P<0.05$) number of lymphocytes existed in the treated animals over the control group. The neutrophils were however higher in the control animals than the test animals.

However, studies on the blood profile of broiler chickens exposed to burning crude petroleum is limited. This study was therefore carried out to determine the haematological status of broiler chickens exposed to refined petroleum products (kerosene) flame and fume emissions.

*Corresponding Author: aoa4u@yahoo.com

Materials and Methods

The experiment was conducted in the Teaching and Research Farm of Rivers State University of Science and Technology, Port Harcourt - Nigeria in an open sided deep litter poultry house. The kerosene was burnt in a designed metal burner measuring 22.86cm high, 17.80cm diameter and a thickness of 1.27cm from 6am - 10pm daily throughout the experimental period of 8 weeks.

One hundred and twenty day-old chicks (Aboika breed) were randomly distributed to 4 treatment groups specified as T1, T2, T3 and T4 (control). Thirty (30) birds were assigned to each treatment, replicated thrice at 10 birds per replicate.

T1 = 4m from the kerosene flame
T2 = 8m from he kerosene flame
T3 = 12m from the kerosene flame
T4 = (Control) located in a different poultry house without kerosene flame.

The birds were fed ad-libitum on a proprietory broiler starter mash containing (2285.6 kcalME/kg and 24.91% crude protein) for 5 week, and a broiler finisher mash (2304.9 kcalME/kg and 20.05% crude protein) for 3 weeks. Water was provided ad libitum and routine inoculation and other medications were administered as and when due.

Blood samples were taken at the 4th and 8th weeks from both the treated groups and the control for haematological assay. Blood collection was by venipuncture with hypodermic needle into well - labeled tubes containing ethylene diamine tetra acetic acid (EDTA) and were taken to the University of Port Harcourt Teaching Hospital (UPTH) Laboratory for analyses. Data obtained on Hb, PCV, WBC, Neutrophils and lymphocytes were subjected to analysis of variance procedure of Steel and Torrie (1980). The means were separated using Duncan's Multiple Range Test (DMRT) as modified by Gomez and Gomez (1984).

Results and Discussion

There were no significant differences ($P>0.05$) in their means among the various treatment groups in respect of haemoglobin, neutrophils and lympocytes in both the brooding (0-5 weeks) and finishing (5-8 weeks) but there were significant differences ($P<0.05$) in the means of packed cell volume (PCV) in the finishing phase (Table 1). This was probably

Table 1: Overall effect of kerosene flame and fumes on broiler chicken haematological characteristics

Week	Treatment	Distance (meters)	Haemoglobin (HB)	Packed Cell Volume (PCV)	White Blood Cell (WBC)	Neutrophils	lymphocytes
4	T1	4	8.96	26.90	7.26a	53.00	46.66
	T2	8	9.76	29.30	8.66b	50.33	47.00
	T3	10	9.90	29.70	9.13c	51.33	47.33
	T4	Control	10.06	30.20	9.90c	49.00	50.33
Standard Error (SE)			± 0.68	± 2.05	± 0.58	± 2.30	± 2.27
8	T1	4	8.80	26.40a	17.33	57.66	39.33
	T2	8	8.40	25.20a	17.33	63.66	35.66
	T3	10	8.50	25.50a	17.66	61.33	36.66
	T4	Control	7.80	23.40b	16.86	60.33	38.00
Standard Error (SE)			± 0.71	± 0.67	± 1.05	± 1.14	± 1.94

a.b.c.d - Means within each column that bear different superscripts differ significantly

due to a raised haemotocrit level which reflects haemo-concentration in the blood.

However, there were significant differences in the means of the white blood cells (WBC) in the brooding phase, indicating increased white blood cells in the blood to maintain homeostatsis for defence mechanism.

In relation to age of the birds, Hb, PCV and lymphocyte decreased with increasing age of birds. WBC and neutrophils increased with increasing age of birds.

Haematological characteristics in animals are known to change with such factors as nutrition, environment, health condition, age, sex, season, strain, breed and physiological state (Saita, 1974). Ingested crude oil in feed showed various levels of toxicity in animals, affecting their blood profile (Ovuru, 2002). Increased crude oil concentration caused leukaemia, erythropenia, neutrophila, lymphocytosis and alteration in platelet morphology (Saita, 1974; Matsumura, 1975; Ngodigha et al., 1999). Haemoglobin, packed cell volume, white blood cells and lymphocyte counts decreased with increasing concentration of crude oil contamination (Ovuru, 2002). Ngodigha et al., (1999) also found a reduction in lymphocyte counts in test goats fed with crude oil contaminated forage. Crude oil has been found to change the blood chemistry and cause antibody depression, lymphatic involution and the release of Adreno-Cortico-Trophic-Hormone (ACTH) and glucocorticoids in stressed animals (Thompson and Lippman; 1974; Siegel, 1980 and Sudakov, 1992). Inhalation of noxious gases such as CO, SO2 and H2S also affect blood chemistry particularly haemoglobin and leucocytes (Uchegbu, 1998; Horsfall and Spiff, 2001). Generally, the blood profile was affected by the inhalation of the kerosene fumes, radiation and light intensity from the flame, resulting in the depressed or lowered blood parameters observed in this study. This might have affected their defence mechanism, as well as predisposing them to anaemia and stress.

Conclusion

The experiment focused on the effect of refined petroleum product (kerosene) flame and fumes on broiler chicken haematological characteristics. The burning of kerosene in both the brooding and finishing phases of broiler chicks resulted in negative responses in the parameters measured. However, kerosene which is used globally for lighting in lamps (illuminant), burning bush, grasses, papers, brooding of day old chicks in stoves, aviation fuel, also has its associated problems of respiratory diseases upon inhalation. The heat (thermal) radiation and continuous light intensity from the burning of kerosene, also added to the stress of the birds, in addition to inhaled fumes. The use of kerosene therefore is not recommended in poultry production especially the brooding phase, in which noxious gases are emitted, VOC and SPM produced by the kerosene flame (as fumes) tended to affect their respiratory systems, liver and heart. Where feasible, electricity as an option should be advocated. But where kerosene has to be used for instance in villages, there should be adequate ventilation to disperse the fumes and heat to reduce the stress level on the chicks.

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References

Ababio O Y. (1993). New School Chemistry for Senior Secondary School. Third Edition. African Feb-Publishers Limited (AEP), Onitsha, Nigeria, pp. 1 12 - 120.

Eniolorunda O O, Awojobi H A and Adeyemi S A. (2005). Haematological studies and Health status of sheep fed varying levels of Spondias mombin leaves plus concentrate. *Trap. J. Ani, Sci.*8 (1): 49-53.

Gomez K A and Gomez A A. (1984). Statistical Procedure for Agricultural Research. 2nd ed. John Wiley & Sons Inc.

Horsfall M and Spiff A I. (2001). Principles of Environmental Pollution (with Physical, Chemical and Biological emphasis) Metroprint Publishers Ltd. Port Harcourt, Chapter 1, pp 1-30.

Igene F U and Arijeniwa A. (2004). The haematological characteristics of growing pigs fed graded levels of cassava peel meal diets. Proc. 29th Ann. Conf., *Nig. Soc. for Ani. Prod. (NSAP)*, 29: 235 - 237.

Jumoke E. (1999). Comprehensive Chemistry for .Senior Secondary Schools. Sure Bet for WASSCE. Glance Series. A. Johnson Publ. Ltd. Ikate, Surulere, Lagos, pp. 95 - 96.

Kaneko J J. (1989). Clinical Biochemistry of Domestic Animals. 4 ed. Academic Press Inc. Saw Santiago, p. 60.

Matsumura F. (1975). Energy of insecticides into animal system. In; Toxicology of Insecticides. Plier. Press, New York, U.S.A. pp 165 - 179.

Murray P R S. (1972) Principle of Organic Chemistry. A Modern and Comprehensive Text for Schools and .Colleges. South East Asian reprint. H. E. B. Hong Kong, Singapore, Kuala Lumpur, London, pp. 92 - 96.

Ngodigha E M, Olayimika F O, Oruwari B M, Ekweozor I K E and Wejdje S N. (1999). Toxic effects of crude oil on organ and blood cells of West African Dwarf Goats. Nig. Vet. J. 20 (1): 82-91.

Ovuru S S. (2002). Gonadal histology and physiological responses of rabbits exposed to crude oil contaminated feeds. Ph.D dissertation. Dept of Anim Sci., Rivers State University of Science and Technology, Port Harcourt, Nigeria.

Owen O J, Alawa J P, Wekhe S N, Aniebo A O, Ngodigha E.M. and A.O. Amakiri (2008). Haematological and biochemical responses of weaner rabbits fed diets containing graded levels of poultry litter. Int. J. of Biotech. and Biochem. (4) 1 pp. 33 – 37.

Saita G. (1974) Benzene induced hypoplastic anaemia and Leukamia in blood disorder due to drugs and other agents. Girdwood, R. A (ed) Excepta media, Amsterdam, Riedel publication pp 127 - 145.

Siegel H S. (1971). Adrenals, Stress and the Environment. World Poultry Science Journal 27: 327-349.

Steel R G D and Torrie J H. (1980). Principles and Procedures of Statistics. McGraw-Hill book Co. inc., New-York, pp. 113 -114.

Sudakov K V. (1992). Stress Postulate: Analysis from the position of general theory of functional system. Pathol. physiol. and Exper. Therapy, 4: 86-93.

Thompson E B and Lippman M E. (1974). Mechanism of action of glucocorticoids. Metabolism, 23; 159-202.

Uchegbu S N. (1998). Environmental Management and Protection, Precision Printer Pub, Enugu. pp 17-35.

Wekhe S N, Yahaya M A, Mgbera O O and Mepba O O. (2001). Leucocytic responses to tolerable level of crude oil ingestion. Proceedings of 26lh Annual Conf Nig. Soc. Anim. Prod. March 16-20, Institute of Agriculture Research and Training, Obafemi Awolowo University, Ibadan Nigeria.

White I C. (1975). Toxicity testing of oil dispersants: aquatic pollution of living resources. Bio-Assay and Toxicity testing Bull. FAO/U.N. pp. 168.

ERRATUM

Dear Readers kindly note the following changes:

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Instead of: Caractérisation des Paramètres de Productivité des Poules Locales (Gallus Gallus Domesticus) de Côte D'ivoire Élevées en Conditions Semi Intensives"
AR Kamga – Waladjo, FJ Mougang, PM Diallo, PEH Diop, D Tainturier

Read: Séroprévalence de la néosporose et conséquences sur la fertilité des vaches laitières à Dakar – Sénégal
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AFRICAN UNION - INTERAFRICAN BUREAU FOR ANIMAL RESOURCES (AU-IBAR)

Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
December 2011

Preamble

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states.

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- Genetic improvement and Biotechnology
- Animal production, nutrition and welfare
- Science and policy in animal health and production
- All aspects of honey bees, especially their social behavior, foraging and use of social and solitary bees for crop pollination activities
- Developments in beekeeping equipment and techniques
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- Global change and wildlife management
- Diseases and their impacts on wildlife populations
- Wildlife management in urban and agricultural environments
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News and announcements: BAHPA is pleased to publish information on animal health and production activities/meetings. Please send the following information to the Editor: Date of the event, title, organization offering the event, location and contact information. Please allow 3 months for the listing to be published.

Submission Guidelines

All manuscripts submitted to BAHPA should include the following features:

1. On page one of the manuscript, the following should be clearly written/inserted: the corresponding author, name of the institution, place where the work was carried out, title of the manuscript, names of the authors, the addresses of the authors and the e-mail address of the corresponding author. The corresponding author should ensure that all the other authors consent to their names being included. The consent should be sent directly by co-authors to the editor via email.
2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion and References.
3. Title, which should be concise, preferably not more than 15 words long, followed by the author(s) name(s) and institution(s) to which work should be attributed and address for correspondence, if different.
4. The Abstract should not be longer than 300 words giving a synopsis of the findings presented and the conclusion(s) reached. Up to six keywords should be provided. The abstract should contain the objectives, brief description of materials and methods, highlights of significant results, conclusions and recommendations.
5. The Introduction should contain the problem statement, the hypothesis and the objective of the work and cite recent important work undertaken by others.
6. Materials and Methods should describe materials, methods, apparatus, experimental procedure and statistical methods (experimental design, data collection and data analysis) in sufficient detail to allow other authors to reproduce the results. This part may have subheadings. The experimental methods and treatments applied shall conform to the most recent guidelines on the animal's treatment and care. For manuscripts that report complex statistics, the Editor recommends statistical consultation (or at least expertise); a biostatistician may review such manuscripts during the review process. Identify the statistical tests used to analyze the data. Indicate the prospectively determined P value that was taken to indicate a significant difference. Cite only textbook and published article references to support your choices of tests. Identify any statistics software used.
7. Results or experimental data should be presented clearly and concisely, in a non-repetitive way. Subheadings may be accepted
8. Discussion of significance should be focused on the interpretation of experimental findings. Subheadings are not accepted in this section
9. State the conclusions, theories, implications, recommendations that may be drawn from the study.
10. Provide a paragraph of around 100 words only, explaining the importance of the manuscript's findings for a non-specialist audience. These points will be published at the end of the article in a box entitled 'Impact'.
11. Short Communications: Manuscripts should contain original data and be limited to 1500 words. The number of tables and figures are limited to two. A limited number of references should be included. Headings are not allowed in short communications.

Sequence of Preparation

1. The data files must be PC/Windows-compatible. The text should be prepared using standard software (Microsoft Word) format; do not use automated or manual hyphenation. Please do not include footnotes.
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3. Use 1 inch margins on top, bottom, left and right margins,
4. Every line on the text should be numbered.
5. Use double space lines spacing for body of text. For Abstract, Figures, Tables and References use single line spacing.
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7. Run "the spell check" and "grammar check" on the entire file before submission.
8. Avoid using abbreviations for the names of concepts. Use ordinary words for variable names – not code names or other abbreviations. Use the same name for a variable throughout your text, tables, figures and appendices. Names of organizations and research instruments may be abbreviated, but give the full name (with abbreviation in brackets) the first time you mention one of these.
9. Acknowledgements of grants and technical help should not be included in the text but at the end after the paragraph Conclusion. Acknowledgements: Under Acknowledgements please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study and any potential conflict of interests if appropriate. Suppliers of materials should be named and their location (town, state/county, country) included.
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Examples of References

- Journal Articles: Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. Journal of American Science, 1(2): 14-21.
- Books: Durbin R, Eddy SR, Krogh A, Mitchison G, 1999.

Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.

- Chapter in a Book: Leach J, 1993. Impacts of the Zebra Mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra Mussels: Biology, Impacts and Control, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- Reports: Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- Conference Proceedings: Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- Thesis: Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- Web links: Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

Illustrations
Please send the figures as separate files and do not import them into the text file. Put all tables, figures, diagrams and artwork on separate pages. Each figure, table, and bibliographic entry must have a reference in the text. References to tables and figures in the text should be by number and not to "table below" or "figure below". The Editor will place them in the appropriate place in the text of article during the final edit. Tables and figures should be numbered consecutively. Please submit the data for figures in black and white.
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Ethical guidelines
BAHPA adheres to the below ethical guidelines for publication and research. Experimentation will only be published if such research has been conducted in full accordance with ethical principles. Manuscripts containing experimentations must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

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