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# IMPACT DES TIQUES SUR DES BOVINS METISSES DANS LE NORD DE LA CÔTE D'IVOIRE

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## Résumé

Le suivi d'animaux métis (N'Dama x Abondance, N'Damance x Montbéliard, N'Damance x Holstein) élevés suivant les pratiques traditionnelles et modernes a permis de recenser les genres et espèces de tiques présentes dans la Région du Poro (Ex-Région des Savanes), dans le Nord de la Côte d'Ivoire ainsi que leurs variations saisonnières. La tique *Amblyomma variegatum* a été majoritaire avec un niveau d'infestation qui a atteint 1200 tiques adultes par animal et était largement supérieur à ce qui est observé chez les races locales. L'impact de la dermatophilose était plus important chez les animaux non traités (plus de 90%) avec des lésions parfois étendues à tout le corps. Les blessures ont surtout été localisées dans les zones de prédilections des adultes d'*Amblyomma* notamment dans la région des mamelles ou du scrotum. D'autres effets néfastes ont été observés dont l'anémie et la perte de poids. L'hématocrite a atteint fréquemment des taux de 15 % avant la mort des animaux. Au niveau clinique, la coudriose n'a été identifiée qu'une seule fois. Les prévalences de *Theileria* spp, *Babesia bovis* et *Babesia bigemina* observés à partir de frottis sanguins étaient respectivement de 5%, 0,1% et 0,05%.

Le rythme de traitement suivi n'était pas adapté aux variations saisonnières des tiques majoritaires, notamment de *Amblyomma variegatum*.

**Mots clés:** Tiques, Variations saisonnières, Bovins métissés, Nord de la Côte d'Ivoire.

## Abstract

Bred mixed animals (Ndama x Abondance (Ndamance), Ndamance x Montbeliard, Ndamance x Holstein) following in traditional and modern herds, allowed us to know the ticks genus and species and their seasonal variation in savannah region of North Cote d'Ivoire.

*Amblyomma variegatum* level of infestation was higher in such breed than local one (up to 1200 adults ticks per animal) and Dermatophilosis impact was important (up to 90% for untreated animals) with lesions, sometimes, extended to all the body. Slight wound was seen in predilections areas of *Amblyomma* adults localized in udder or scrotum regions. Others adverse effects were observed as anemia and weight loss. The PVC value was frequently observed before animal's death. At clinical level, Heartwater was identified only once. The prevalence of *Theileria* sp, *Babesia bovis* and *B. bigemina* was observed from blood smears were 5%, 0.1% and 0.05% respectively. The rate of follow up treatment rate was not adapted to seasonal variation of ticks, including *Amblyomma variegatum*.

**Key Words:** Ticks, Seasonal variation, Mixed breed cattle, North Côte d'Ivoire

## Introduction

Le secteur élevage en Côte d'Ivoire, contribuait pour environ 2,9 % au PIB agricole et pour environ 1% au PIB total avant les événements sociopolitiques de 2002. Il constitue néanmoins une activité importante qui concourt à l'accroissement de la sécurité alimentaire, à la diversification des sources de revenus pour les éleveurs ainsi qu'à l'accroissement de la productivité agricole à travers l'intégration agriculture-élevage. Il assure ainsi la subsistance d'une partie importante de la population rurale du Nord de la Côte d'Ivoire.

Le pays possédait un cheptel bovin d'environ 1 500 000 têtes en 2001 (DPP, 2001). Les types génétiques rencontrés sont les taurins et les zébus. Les taurins sont représentés par les races N'Dama, Baoulé et lagunaires. Compte tenu du format très réduit des taurins, les paysans pratiquent depuis longtemps des métissages avec le Zébu. Dans le souci de réduire la dépendance du pays vis-à-vis de l'extérieur en matière de viande, il fut créé en 1979, dans le Nord du pays, un projet intitulé Bovins Industriels (BI) avec deux stations dont l'une à Panya, dans le département de Boundiali et l'autre à Nioroningué, dans le département de Ferkessédougou. Ce projet avait pour objectif de faire surtout des croisements entre la race européenne Abondance et la race locale N'Dama dans le but de les diffuser auprès des éleveurs pour augmenter leur production en lait et en viande. Les produits ainsi obtenus furent appelés N'Damance.

Depuis 1995, le projet Bovins Industriels a été renommé Projet National d'Appui au Développement Laitier (PNADEL) avec pour objectif l'augmentation du potentiel laitier de la N'Damance. Les femelles sont inséminées avec de la semence de taureaux des races Montbéliarde et Holstein.

Le climat tropical de l'ensemble du pays est un facteur favorable au développement de nombreuses maladies parasitaires d'incidences économiques importantes auxquelles il faut ajouter une alimentation défectueuse pendant la saison sèche. Pour cette raison, les animaux métis, ainsi produits, font l'objet de soins particuliers surtout en ce qui concerne les

tiques.

En effet, de fortes mortalités liées aux tiques ont été signalées sur des animaux de races exotiques et des métis au Cameroun (21), au Ghana (13,17) et au Nigeria (14). En Côte d'Ivoire, les seules données exhaustives sur les tiques sont celles des travaux d'Aeschlimann (1) et Morel (16) qui datent des années 1950 à 1970.

L'objectif principal de cette étude est d'estimer l'impact des tiques sur les nouveaux types génétiques de bovins. Il s'agira dans un premier temps de faire un inventaire des tiques présentes, leur zone de prédilection et leur saisonnalité. Dans un second temps, il faudrait déterminer la sensibilité de ces animaux croisés aux tiques, la prévalence des maladies transmises par ces tiques et estimer leur productivité.

## Materiel et Methode

### Zone d'étude

La Région du Poro, ex-Région des Savanes, dans le Nord de la Côte d'Ivoire, a un relief caractérisé par une succession de collines et de plaines avec une dominance de plateaux dont les altitudes varient de 300 à 500 mètres. Elle est composée de plus de 80 % de formations savaniques qui sont la savane boisée, la savane arborée, la savane arbustive et la savane herbeuse.

Le climat est de type soudanien avec une saison unique des pluies qui dure six mois et demi (mi-avril à octobre), avec un pic de juillet à octobre; une saison sèche dont l'influence maximale se situe de novembre à fin mars.

La région Nord a une pluviométrie moyenne annuelle variant entre 1200 et 1500 mm (intervalle 800 à 2100 mm). Les températures moyennes de la région sont en général supérieures à 24° C (minima en janvier 15°C ; maxima en mars 40° C).

Les systèmes de production bovine rencontrés dans le Nord de la Côte d'Ivoire sont l'élevage sédentaire (43%) et l'élevage transhumant (50%). Les troupeaux sédentaires comportent par ordre d'importance décroissante les races Baoulé, Zébu, N'Dama, métis Zébu-Baoulé et autres métis. Le cheptel

transhumant est composé essentiellement de zébus (63%). Toutefois, le bétail Baoulé occupe une place non négligeable (13%) ainsi que les métis Zébu x Baoulé (21%). La culture du coton a favorisé l'émergence des bœufs de culture attelée qui représentent environ 7% du cheptel du Nord.

L'alimentation des animaux est constituée essentiellement de fourrages naturels. Dans les fermes d'Etat, des pâturages artificiels de type panicum ont été mis en place pour accroître les ressources fourragères. Au cours de la saison sèche, les animaux reçoivent une complémentation avec des sous produits agricoles constitués majoritairement de graines de coton.

Sur le plan sanitaire, des programmes de traitements systématiques existent et portent sur les parasites gastro-intestinaux, les trypanosomes et les tiques. Le plan annuel préconisé par l'ex-Société de Développement des Production Animales (SODEPRA) pour la lutte contre les tiques s'établit comme suit :

- deux détiquages mensuels en saison sèche (décembre à mars) ;
- deux détiquages mensuels et en début de saison de pluies (avril à mai) ;
- trois à quatre détiquages en saison des pluies (juin à octobre) ;
- deux à trois détiquages en début saison sèche (octobre à novembre).

Les acaricides utilisés sont essentiellement l'Amitraze et les pyrèthrinoides. La pulvérisation est le mode d'application le plus répandu. Les bains et les douches sont pratiqués dans les ranches et les fermes d'Etat.

#### *Animaux*

Les animaux suivis, ont été en majorité des métis Montbéliard et Holstein. Ainsi, la première année, sept troupeaux étaient répartis dans trois fermes (Nioroungué, Djibé, Badikaha) de la manière suivante :

**Groupe 1** : dit témoin (non détiqué) de 15 taurillons à la station de Nioroungué

**Groupe 2** : constitués de 4 sous-groupes de 10 animaux chacun à Nioroungué (N1, N2, N3, N4) traités selon le protocole en vigueur à la station, de 15 animaux dans la ferme privée

de Djibé et de 15 animaux de la ferme privée de Badikaha. Les animaux des fermes privées ont été détiqués selon leur propre protocole et selon la disponibilité des produits.

Pendant la seconde année, deux groupes ont été constitués dans la station d'Etat :

- Un lot (T9) qui a suivi le traitement selon le protocole de l'ex-Sodepra ;
- Un lot (T10) a suivi un protocole réduit (Un traitement tous les quinze jours en saison des pluies).

#### *Comptage des tiques*

Sept régions anatomiques ont été définies. Ce sont: la région anale et la queue, la région mammaire ou du scrotum, le ventre, l'aisselle, le fanon, la tête et les pattes. Dans chaque site, les tiques ont été récoltées et comptées sur la moitié du corps des animaux puis conservées dans un bocal contenant de l'alcool à 70°. L'identification des genres a été faite sur le terrain et celle des espèces au laboratoire. Chez les animaux témoins, les tiques ont été comptées sans être récoltées. Le chiffre obtenu a été multiplié par deux pour avoir le nombre total de tiques par animal. Le rythme de passage est d'une fois tous les quinze jours.

#### *Hématocrite et frottis sanguins*

La mesure de l'hématocrite des animaux a été faite sur le terrain. Les frottis sanguins ont été confectionnés puis fixés au méthanol, colorés au Giemsa et observés au microscope pour la recherche de parasites sanguins (2).

#### *Analyses complémentaires*

Les lésions dermatologiques ainsi que les autres signes cliniques observés sur les animaux ont été notés. Le poids des témoins a été relevé tous les 15 jours tandis que celui des autres animaux était mesuré mensuellement. La température rectale a été aussi prise sur les témoins présentant un état général altéré.

Des écrasements de cortex cérébral ont été confectionnés sur des cadavres et examinés au microscope pour la recherche d'amas d'Ehrlichia ruminantium.

Analyses statistiques

Les données ont été saisies, vérifiées et analysées sur le logiciel Epi-Info. La plupart des variables étudiées n'étant pas normalement distribuées, une analyse non paramétrique a été privilégiée. Des comparaisons entre plusieurs groupes ont été faites à l'aide de la méthode de Kruskal-Wallis. Le seuil de signification a été fixée à  $p < 0,05$ .

Resultats

Sauf indications contraires, les données présentées ci-dessous sont la compilation des données observées sur les sept troupeaux.

Genres et espèces de tiques présentes dans la région.

Quatre genres de tiques ont été rencontrés (Tableau I). La tique Amblyomma, la plus prédominante, représentait 66 à 90% des tiques récoltées selon les fermes. Elle est suivie par Boophilus (8 à 33%) puis Hyalomma (0,5 à 3%) et enfin Rhipicephalus (moins de 1%). Leur importance dans les troupeaux suivis est représentée dans la figure 1.

Le genre Amblyomma est représenté par une seule espèce, Amblyomma variegatum. Deux espèces de Boophilus ont été identifiées, ce sont B. decoloratus et B. geigy. Deux espèces de Hyalomma qui sont, H. marginatum rufipes et H. truncatum et une espèce de Rhipicephalus (Rh. lunulatus) ont été identifiées.

Variations saisonnières des tiques.

Les variations saisonnières de Amblyomma ont été étudiées (Figure 2). Pour Boophilus, les adultes et les nymphes étaient présents quelque soit la saison et les larves, peu visibles en raison de leur petite taille, se

rencontraient occasionnellement. Pour ce qui concerne Hyalomma et Rhipicephalus, seuls les adultes ont été trouvés sur les animaux.

Les différents stades de Amblyomma variegatum se succédaient au cours de l'année. Les mâles étaient présents de mars à juillet avec un maximum en mai. Ils étaient rares, voire inexistant, de septembre à février. Les femelles étaient rares de novembre à avril et atteignaient leur nombre maximal en juin. Les larves disparaissaient de février à septembre. Néanmoins dans certains troupeaux, quelques larves ont été rencontrées en mars, avril et même en mai. Le pic d'infestation par les larves a été observé en octobre. Les nymphes ont fait leur apparition d'octobre à mai avec un maximum en décembre ou janvier selon les fermes.

Les Boophilus ont été rares de juin à septembre. Les Hyalomma ont été présents toute l'année et les Rhipicephalus ont été rencontrés de mai à juillet.

Zones de prédilection

Pour le genre Amblyomma, les sites préférentiels des adultes étaient la région mammaire, l'aisselle, le ventre et le fanon (Figure 3). Les nymphes avaient pour sites préférentiels, l'aisselle, la région mammaire, le ventre et les pattes. Les larves avaient leurs sites de prédilection sur la tête, le fanon, les pattes et l'aisselle (Figure 3).

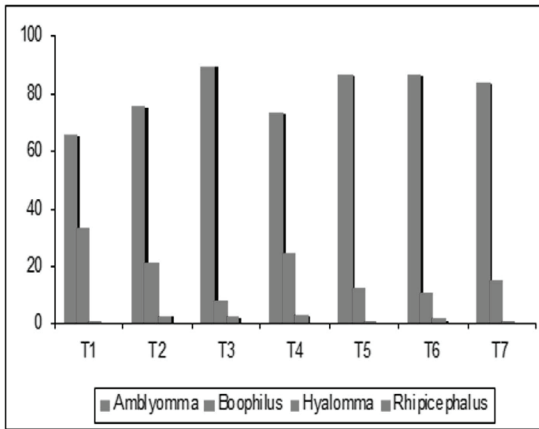
Les sites de prédilection du genre Boophilus étaient le fanon, le ventre et la tête (Figure 4). Les Hyalomma se sont fixés préférentiellement aux régions anale et mammaire et Rhipicephalus au niveau des pattes.

Pathogènes transmis par les tiques

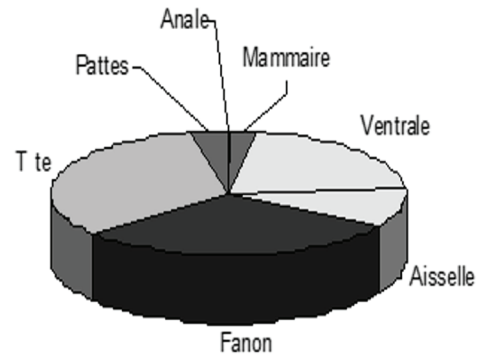
Sur 2515 frottis examinés, seuls 132 (5,25%) ont été positifs à Theileria sp, 3 (0,12%) à Babesia bovis et 1 (0,04 %) à Babesia bigemina. Dans les troupeaux détiqués, il n'y avait pas de relation entre la présence de Theileria et l'état de santé des animaux. Par contre, dans le troupeau témoin, une forte corrélation ( $r=0,86$ ) statistiquement significative ( $p < 0,05$ ) a été notée entre les animaux porteurs de Theileria et les animaux fortement infestés (Figure 5).

Tableau I : Les genres et espèces de tiques des bovins du nord de la Côte d'Ivoire.

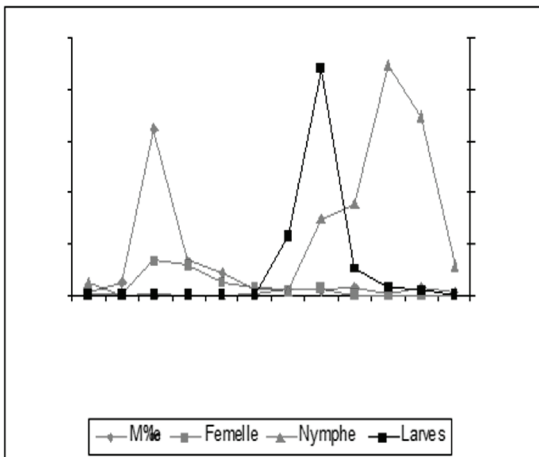
Genres	Espèces
Amblyomma	- variegatum
Boophilus	- geigy - decoloratus
Hyalomma	- marginatum rufipes - truncatum
Rhipicephalus	- lunulatus



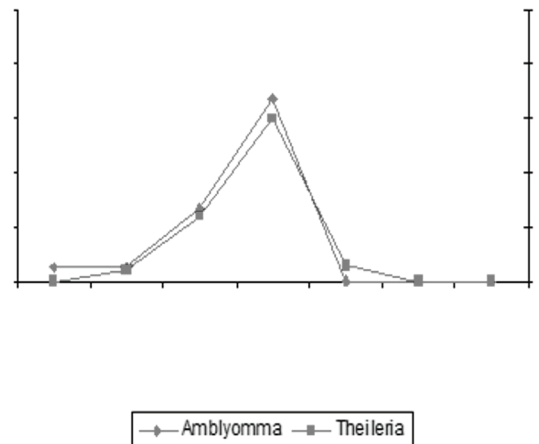
**Figure 1 :** Importance des 4 genres de tiques dans les troupeaux suivis.



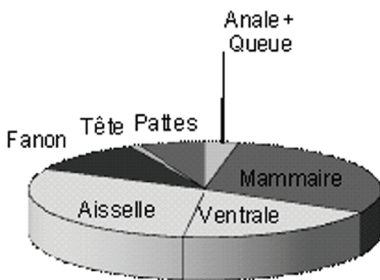
**Figure 4:** Sites de fixation du genre *Boophilus* sur les animaux.



**Figure 2 :** Variation saisonnière d'*Amblyomma variegatum* : cas du troupeau de Djigbè.



**Figure 5:** Relation entre l'intensité d'*Amblyomma variegatum* et l'infestation par *Theileria* spp.



**Figure 3:** Sites de prédilection des adultes d'*Amblyomma* sur les animaux.

#### Intensité d'infestation

Dans les troupeaux où les traitements acaricides étaient appliqués, 50 à 75 % des animaux hébergeaient moins de dix *Amblyomma variegatum*. Une forte infestation a néanmoins été observée dans les fermes privées de Djigbè et Badikaha.

Dans le troupeau témoin, des niveaux d'infestation élevés ont été atteints. Les métis Holstein se sont révélés plus sensibles avec un nombre moyen de tiques (1200) supérieur à celui des métis Montbéliarde (600) mais cela n'a pas été statistiquement significatif.

#### Signes cliniques et lésions

Les animaux sont restés en bon état général durant le premier mois. Les signes

cliniques qui ont été observés sur tous les animaux non détiqués étaient les suivants : l'apathie, l'anorexie, la fièvre, l'amaigrissement, l'anémie. Des maladies telles que la dermatophilose, l'orchite, la parakératose et les myiases, ont été observées chez ces animaux.

La dermatophilose est apparue dans la deuxième moitié du mois de mai (début saison des pluies) et a été observée sur la quasi-totalité des animaux dont l'état général s'était de plus en plus altéré. Vers la fin du mois de mai, elle s'est aggravée par les myiases chez les animaux témoins. Les lésions étaient d'abord localisées à la région du scrotum ou mammaire et se sont ensuite généralisées, très rapidement, à l'ensemble du corps.

La dermatophilose a été observée chez 93% des animaux témoins et chez 40 à 60% des animaux détiqués de la station Niorouingué. La gravité des lésions est fonction non seulement de l'intensité de l'infestation par les tiques mais aussi de la race de l'animal (Les Holstein étaient les plus atteints). Dans les élevages privés, cette affection a touché 23% des animaux à Badikaha et 43% à Djigbè.

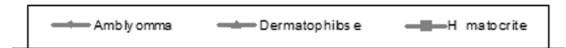
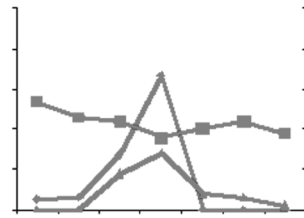
Des zones de parakératose ont été observées chez des animaux guéris de dermatophilose et étaient localisées essentiellement dans la région mammaire, à l'aisselle et aux flancs.

Les animaux ont été aussi progressivement anémiés. Les corrélations ( $r = -0,141$ ) entre l'hématocrite et les différentes stases de *Amblyomma variegatum* ont été très significatives ( $p < 0,001$ ), indiquant la baisse de l'hématocrite quand le nombre de *Amblyomma* augmente. De même, le nombre de cas de dermatophilose est en corrélation positive avec l'abondance en *Amblyomma* ainsi, plus le nombre de *Amblyomma* augmente, plus des cas de dermatophilose apparaissent (Figure 6).

#### Pertes directes

Les pertes directes observées dans le lot témoin sont :

- La perte de poids : la perte de poids a débuté à la mi-avril. L'amaigrissement a été perceptible au mois de mai où la chute moyenne de poids avoisinait 30 kilogrammes avant de s'aggraver en juin.



**Figure 6:** Relation entre niveau d'infestation par *Amblyomma variegatum*, la dermatophilose et l'hématocrite chez les animaux témoins.

La perte de poids n'a pas été observée dans les troupeaux où le détiquage se pratiquait. Chez ces derniers, il y a plutôt eu un gain de poids;

- L'hyperthermie : la période d'hyperthermie précédant la mort a duré de 8 à 21 jours.
- La mortalité : une forte mortalité (13 sur 15 animaux) a été enregistrée dont 4 en juin, 7 en juillet, 1 en août et 1 en octobre. Le dernier comptage de tiques, sur des animaux morts en juin, a montré des nombres de *Amblyomma variegatum* adultes allant de 830 à 1674.

#### Cowdriose

Sept têtes d'animaux morts ont pu être acheminées au laboratoire pour la recherche d'amas d'*Ehrlichia ruminantium*. Deux animaux se sont révélés négatifs et cinq étaient positifs. Le Centre International de Recherche-Développement sur l'Élevage en zone Subhumide (CIRDES) a confirmé la positivité d'un écrasement, les autres ayant été jugés difficilement lisibles.

#### Effet du rythme des traitements acaricides

Chaque ferme adopte sa propre méthode de lutte contre les tiques.

- A Badikaha, le même rythme de traitement est appliqué à toutes les races présentes. Une rupture d'approvisionnement en produit acaricide de traitements pendant la saison sèche (mi-janvier à avril), ne s'est pas traduite par l'apparition de plus fortes intensités parasitaires aussi bien chez les

rares locales que chez les métis. Le manque de traitement acaricide a eu un effet sur la charge de *Boophilus*.

- A Djigbè, le détiqage pendant la saison sèche suivait le rythme régulier d'une fois par quinzaine. Les animaux sont tous des métis Montbéliard. Le nombre total de nymphes de *Amblyomma* par animal et par passage n'est pas très différent de celui observé à Badikaha où les animaux n'ont pas reçu de traitement.
- A la station de Nioroungou, au cours de la deuxième année, deux troupeaux suivant des rythmes de traitement différents ont été constitués pendant la saison des pluies. Le troupeau T9 suivait le rythme normal qui est celui d'un passage hebdomadaire au bain et le troupeau T10 a suivi un rythme réduit équivalant à un traitement tous les quinze jours. Une différence significative n'a pas été observée au niveau d'infestation dans les deux groupes.

### Discussion

L'étude a permis de déterminer les tiques présentes dans la Région du Poro du Nord de la Côte d'Ivoire ainsi que leur saisonnalité. Les genres correspondent à ce qui est rencontré dans la plupart des pays de la sous-région. La différence réside au niveau des espèces qui ne sont pas toutes présentes dans chaque pays. La variation saisonnière, surtout pour *Amblyomma variegatum*, la tique prédominante a montré aussi l'existence d'une seule génération annuelle comme au Burkina (11) et au Sénégal (8, 9) avec cependant un décalage dans le moment du pic d'infestation par les adultes lié certainement à la précocité ou non d'apparition des pluies. Dans le Centre de la Côte d'Ivoire, Knopf et collaborateurs (12) ont trouvé des adultes *Amblyomma* toute l'année avec cependant des pics au début de la saison des pluies, d'avril à juin.

Dans la station d'Etat, de fortes infestations ont été observées aussi bien chez les animaux régulièrement détiqués que chez les témoins (jusqu'à 1 000 tiques par animal, quatre mois seulement après l'arrêt des détiquages). Chez ces derniers, le niveau d'infestation a été surestimé car les mâles *Amblyomma* peuvent

rester fixés jusqu'à 6 ou 8 mois sur un bovin et la durée moyenne d'engorgement des femelles varie entre 8 et 10 jours (3, 18, 24). Néanmoins, les très forts niveaux d'infestations des témoins montrent que ces traitements réguliers n'ont absolument pas contribué à la diminution du niveau d'infestation des pâturages de la station. Le maintien si élevé de l'infestation des pâturages pourrait s'expliquer de plusieurs manières :

- Le calendrier de la lutte contre les tiques qui a été établi n'a pas été suivi rigoureusement. En effet, dans les stations d'Etat, les traitements étaient liés à la disponibilité des crédits alloués. Dans les fermes privées, les traitements se font au rythme des visites et des moyens financiers du propriétaire ainsi que du degré de communication entre ce dernier et le bouvier.
- La proximité de faune sauvage, constituée d'hôtes alternatifs pour les adultes de *A. variegatum* (phacochères, antilopes, buffles) a pu contribuer à maintenir le niveau élevé de l'infestation des pâturages par la tique.
- La mauvaise application des produits. Dans les fermes privées, le traitement se fait par pulvérisation. Il est possible que le produit ait été mal appliqué aux creux des aisselles et la région inguinale qui sont des sites de prédilection des adultes, ou sur la tête (zone de fixation des larves).
- L'inefficacité de certains acaricides. En effet, des éleveurs pensent que certains produits (surtout les moins chers sur le marché) ne sont pas efficaces à la dilution conseillée par le fabricant et ils sont tentés d'en augmenter la concentration. C'est le cas d'un acaride à base d'alphacyperméthrine dont la dilution préconisée (1ml dans 2 litres d'eau) est utilisé à la dose de 90 ml/ 70 l d'eau, soit environ le triple de la concentration initiale. Même si les pyréthrinoides ont, selon Barré et collaborateurs (4), une faible toxicité vis à vis des mammifères et de l'environnement, le problème d'inefficacité des acaricides devrait être élucidé car nous pourrions être en présence d'une éventuelle résistance aux acaricides. En effet, le mode d'application manuelle des acaricides serait

un facteur favorisant le développement de résistance chez les tiques (6, 20).

Selon Stachurski (22), la présence de *Amblyomma variegatum* dans une région est un obstacle à l'introduction de races améliorées car ceux-ci sont très sensibles à la coudriose et à la dermatophilose. En revanche, les bovins locaux sont généralement immunisés très tôt contre la coudriose (stabilité enzootique) et souvent peu sensibles à la dermatophilose.

Notre étude a montré, un faible taux d'infestation des animaux par des agents pathogènes transmis par les tiques. De faibles taux d'agents pathogènes détectés par la méthode de frottis sanguins ont été aussi rencontrés en Gambie (15), au Nigeria (2) et au Sénégal (9). La cause des mortalités observées dans le lot témoin est plus probablement l'anémie et la dermatophilose. Cette étude a pourtant été initiée à la suite d'une suspicion de coudriose chez des veaux qui mouraient après des troubles nerveux. C'est pourquoi, malgré le seul cas confirmé positif à la coudriose sur les sept lames d'écrasement de cortex cérébral, l'impact de la coudriose mériterait d'être approfondi par une autre étude, avec des méthodes de diagnostic plus sensibles. L'âge des animaux et la période ont dû avoir aussi une influence sur les résultats car le travail n'a pas été mené sur des veaux mais plutôt sur des animaux de 20 à 30 mois qui ont déjà eu une saison de pâture et qui seraient donc immunisés. Le seul test sérologique disponible à l'époque de l'étude ne pouvait malheureusement pas montrer la stabilité enzootique chez les bovins (sensibilité insuffisante, Stachurski, communication personnelle). La méthode sérologique développée par le CTVM d'Edinburgh (MAPI-B ELISA) et testée au Ghana par Bell-Sakyi et collaborateurs (5) aurait permis cette étude, mais la sérothèque a disparu avec les événements de 2002. S'il s'avérait que la coudriose était endémique, alors les rythmes de détiqage suivis jusqu'à là auraient alors une base scientifique pour être fortement revus à la baisse, notamment en saison sèche. En effet, dans le troupeau où il n'y a pas eu de traitements acaricides de la mi-janvier à la mi-avril par manque de produit, le niveau d'infestation a été aussi bas que celui dans

lequel le traitement a été fait selon le rythme de la saison à savoir un détiqage tous les 15 jours. La saison sèche correspond à la période de la présence des nymphes dont la durée moyenne d'engorgement est de 6 jours (22). Ce qui laisse supposer que l'efficacité d'un traitement en saison sèche dépend de la rémanence du produit utilisé. Avec ce rythme de traitement, les produits qui ont une rémanence de 5-7 jours n'empêchent pas la fixation des nymphes, encore moins la transmission de pathogènes. Cette situation nous permet de nous interroger s'il était encore opportun de détiqer en saison sèche. Mais le manque de traitement en saison sèche a eu pour conséquence l'augmentation du niveau d'infestation des *Boophilus*. Or tout comme les *Amblyomma*, les fortes infestations de *Boophilus* ont un effet néfaste sur les performances laitières et le poids des animaux (7, 10, 19, 21, 23). Une femelle *Amblyomma* peut en plus faire perdre jusqu'à 20 ml de sang à l'animal (25). Un équilibre devrait donc être trouvé pour la lutte contre ces deux tiques majeures du bétail en fonction de l'objectif de production visé.

L'étude a aussi montré que l'intervalle entre les traitements devrait être augmenté pendant la saison des pluies. En effet, un traitement hebdomadaire est valable au début et à la fin de la saison pluvieuse quand les pluies sont encore espacées. Au moment des fortes pluies par contre (août, septembre et octobre), un traitement tous les quinze jours donne des résultats similaires à un traitement tous les 8 jours. Les traitements du début de la saison des pluies rejoignent ceux qui sont conseillés aux éleveurs de bovins de races locales autour de Bobo-Dioulasso. Avec la coudriose qui était non endémique dans cette zone, un nouveau rythme de traitement devrait être envisagé surtout pour les animaux métissés. Les élevages privés et plus particulièrement les fermes d'Etat sont concernés par cette limitation du nombre d'intervention établis par mimétisme par rapport à la situation qui prévaut dans les régions où des fortes mortalités liées aux tiques sont observées sur des races exotiques. Ceci aurait au moins l'avantage de minimiser les coûts des intrants liés aux traitements acaricides.

Vouloir faire des économies sur les acaricides est une chose, mais il ne faut pas perdre de vue que nous sommes en présence d'animaux métissés supposés être sensibles aux tiques et aux agents pathogènes transmis par les tiques. En effet, dans la ferme privée où sont élevés ensembles des animaux de races locales et des animaux métissés avec des races exotiques européennes, ces derniers se sont montrés plus sensibles à l'infestation des tiques en certaines périodes de l'année. C'est pourquoi les perspectives qui s'ouvrent à cette étude sont d'une part la recherche approfondie sur la stabilité enzootique ou non de la cowdriose et, d'autre part l'évaluation de schémas de traitements réduits et l'élucidation de la perception de résistance aux acaricides.

Cette étude permettra aux éleveurs d'améliorer le traitement aux acaricides, permettant ainsi d'augmenter la productivité du bétail et leur condition de vie.

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## ACUTE RESPIRATORY DISEASE ASSOCIATED WITH *MANNHEIMIA HAEMOLYTICA* INFECTION IN A HERD OF WEST AFRICAN DWARF SHEEP.

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### Abstract

An acute severe fibrinonecrotic bronchopneumonia suggestive of *Mannheimia haemolytica* infection was diagnosed histopathologically in West African Dwarf (WAD) sheep submitted to the Veterinary Teaching Hospital (VTH), University of Agriculture, Abeokuta, Nigeria. *Mannheimia spp* was isolated from the nasal swab and lymph node and lung samples of the affected sheep. The isolated organism was found to have cultural, morphological and biochemical properties consistent with those of *Mannheimia haemolytica* biotype A. The organism was resistance to ampicillin, streptomycin, gentamycin, oxytetracycline and trimethoprim in agar disc diffusion method. It was however sensitive to furazolidone, ciprofloxacin and norfloxacin. The present finding is consistent with the view that *M. haemolytica* may occur in a proportion of small ruminants that exhibit respiratory problem. It is therefore suggested that in the present natural outbreak, climatic condition, environmental and/or transportation stress could have resulted in the break down of the defense barrier of the sheep resulting in eventual susceptibility to infection by *Mannheimia haemolytica*.

**Keywords:** Organ pathology, *Mannheimia haemolytica* biotype A, Acute fibrinonecrotic bronchopneumonia, WAD sheep.

## MALADIE RESPIRATOIRE AIGUE ASSOCIEE A L'INFECTION PAR *MANNHEIMIA HAEMOLYTICA* DANS UN TROUPEAU DE MOUTONS NAINS D'AFRIQUE DE L'OUEST.

### Résumé

Une bronchopneumonie fibrinonécrotique aiguë sévère, évocatrice d'une infection à *Mannheimia haemolytica*, a été diagnostiquée par examen histopathologique chez des moutons nains d'Afrique de l'Ouest (WAD : West African Dwarf) soumis à l'Hôpital d'enseignement vétérinaire (VTH) de l'Université d'Agriculture d'Abeokuta au Nigeria. *Mannheimia spp* a été isolée dans des écouillons nasaux et des ganglions lymphatiques et des échantillons pulmonaires des moutons affectés. On a découvert que l'organisme isolé avait des spécificités de culture et des caractéristiques morphologiques et biochimiques correspondant à celles de *Mannheimia haemolytica* biotype A. L'organisme a montré une résistance à l'ampicilline, à la streptomycine, à la gentamycine, à l'oxytétracycline et au triméthoprim dans la méthode de diffusion en gélose. Cependant, l'organisme a montré une sensibilité à la furazolidone, à la ciprofloxacine et à la norfloxacine. Le présent résultat concorde avec l'opinion selon laquelle *M. haemolytica* peut être présente dans une proportion de petits ruminants ayant un problème respiratoire. Il est donc sous-entendu que, dans les épidémies actuelles survenant naturellement, les conditions climatiques, le stress environnemental et / ou le stress du transport ont probablement été à l'origine de la dégradation de la barrière de défense des moutons, avec comme conséquence une sensibilité éventuelle à l'infection par *Mannheimia haemolytica*.

**Mots-clés:** Pathologie des organes ; *Mannheimia haemolytica* biotype A ; Bronchopneumonie fibrinonécrotique aiguë ; Moutons nains d'Afrique de l'Ouest.

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## Introduction

In Nigeria, the ruminant industry suffers high frequency and incidence of respiratory diseases which consequently affect production and productivity. Pneumonia has been recognized as one of the most common respiratory problems in ruminants throughout the world (Martin, 1996; Brogden *et al.*, 1998). *Mannheimia haemolytica* which is more frequently isolated from such cases of pneumonia (Davies, 1985) has been identified as a leading cause of mortality and morbidity in small ruminants and feedlot cattle where it often results in significant economic losses to the producers (Whiteley *et al.*, 1992).

*Mannheimia haemolytica*, a gram-negative and opportunistic pathogen (Bisgard, 1993) has long been recognized as one of the normal flora of the respiratory tract and mouth of many animals. The organism is frequently isolated from asymptomatic carriers (Gilmour and Thompson, 1994; Gilmour and Gilmour, 1989) and apparently healthy sheep (Poulsen *et al.*, 2006). However, the damaging effects of climatic changes, severe transportation or shipping stress and/or viral infection have been incriminated as important predisposing factors which make the organism acquire the ability to rapidly replicate and proliferate in the upper respiratory tract from where it is aspirated into the lungs to produce the characteristic lesion of fibrinonecrotic pleurobronchopneumonia (Jubb *et al.*, 1985). In the lung, the organism is reported to express various identified virulence factors including a ruminant-specific leukotoxin, lipopolysaccharide, a sialoglycoprotease, a neuraminidase and immunoglobulin proteases (Whiteley *et al.*, 1990; Adamu, 2007). Two potent toxins, leukotoxin and lipopolysaccharide have been shown to be the two major virulent factors related to the pathogenicity of the disease and promote inflammatory process in the lungs (Achermann and Brogden, 2000). This has been shown to be due to the fact that the organisms promote the release and expression of cytokines and proinflammatory mediators (Whiteley *et al.*, 1990; Kumar *et al.*, 1991) which contributes to development of fatal fibrinous pleuro-pneumonia in the affected animals (Shiferaw *et al.*, 2006; Poulsen

*et al.*, 2006).

While *M. haemolytica* had been reported and isolated from various animal species (Dviva and Monhan, 2000, Odugbo *et al.*, 2003) and poultry in Nigeria (Antiabongi *et al.*, 2005), the reports of natural mannhheimosis in Nigerian sheep is quite scanty in literature (Odugbo *et al.*, 2003).

Hitherto, there is paucity of reports on gross and histopathology of this disease and in addition, the authors are unaware of any previous report on specific organ pathology in naturally infected animals from established outbreaks. This paper therefore describes the organ pathology associated with *M. haemolytica* infection in a herd of WAD sheep and the antibiotic sensitivity of the bacterial isolate.

## Material and Methods

### Case history

Sixty (60) WAD sheep recently purchased from nearby villages and meant for a nutritional performance experiment were kept together for acclimatization at Alabata village, Abeokuta for a few days before being transferred to the experimental site at the University farm. Five (5) days after, thirty six (60%) out of these sheep were observed to have diarrhea, bilateral mucopurulent nasal discharge average rectal temperature and respiratory rate of 40.3°C and 16 beats/minute respectively. Six (10%) sheep were reported dead the following day and were presented for postmortem examination. In all, eighteen (48%) died within the eight (8) period of the outbreak.

Carcasses were promptly necropsied. Samples from the mandibular lymph nodes and lungs were harvested and preserved for bacteriological examination. Sections from the lungs, liver, kidneys, spleen and brain were also fixed in 10% buffered formalin and later processed routinely for histopathological examination. Tissues were sectioned at 5µ thickness and stained with haematocrylin and eosin (Humason, 1979).

### Bacteriological examination of the sample

Samples of the lymph node and lung tissue collected from the five carcasses were

macerated using sterile pestle and mortar; the homogenate and nasal swabs collected from 25 live sheep were plated on sheep blood agar (Oxoid) and incubated aerobically at 37°C for 24 hours. Small colonies with little central thickening and surrounded by a zone of  $\beta$ -haemolysis were sub-cultured on very young sheep blood and MacConkey agar plate and incubated for 24 hours. Smears prepared from the colonies of the organism on the blood agar plates were stained by Gram's Method (Barrow and Felthan, 1993) and examined under the oil immersion objective ( $\times 100$ ) of a light microscope (Olympus, Germany). The isolated bacterium was examined for motility, acid and gas production from sugars (glucose, maltose, sucrose and trehalose), indole production and oxidase and catalase activities according to the methods of Barrow and Felthan (1993).

The susceptibility of the bacterial strain was observed against eight different antimicrobial agents by agar disc diffusion method described by Mevius and Hartman (2000). The tested antimicrobial agents were amoxicillin (10  $\mu$ g), furazolidone (50  $\mu$ g), ciprofloxacin (10  $\mu$ g), and oxytetracycline (30  $\mu$ g). This was for the purpose of choosing a suitable antimicrobial for the treatment of the remaining forty two sheep.

## Results

### *Clinical and Post-Mortem Findings*

Several sheep (about 75%) were depressed and/or recumbent, had bilateral mucopurulent nasal discharge and high body temperature. All the 5 carcasses posted were moderately emaciated, dehydrated and had rough hair coat. The perineal region in all the carcasses was soiled with diarrhoeic faeces. The mucous membranes of the buccal cavity and conjunctivae were moderately pale. Frothy exudate was present in the trachea, bronchi and bronchioles while the mucosa of the respiratory tract was reddened and showed areas of ecchymotic haemorrhages. The pleurae of the lungs were thickened and showed adhesion, while the lungs were found to be markedly congested and edematous. The middle and apical lobes of the right lungs in all the five carcasses showed severe consolidation,

this involved the anterior and ventral parts; an average of 20% of the lung (15-25% in each sheep) was affected. The degree of consolidation expressed as a percentage of the total lung volume was estimated as described by Odugbo, *et al.*, (2004). The liver was slightly yellowish. The spleen and lymph nodes were slightly enlarged and haemorrhagic. The small intestine contained mucous exudate while the mucosal fold of the colon and rectum was congested and had stripes of ecchymotic haemorrhages. The Peyer's patches were severely swollen and haemorrhagic. Morphological diagnoses of acute pneumonia (severe, cranioventral), fatty change (moderate, diffuse), catarrhal enteritis, congested and haemorrhagic colitis were made. Tentative diagnosis of *Mannheimia* pneumonia was made based on the above lesions while Peste des Petits ruminants (PPR) was queried.

### *Histopathology*

The lung tissue showed vascular congestion and deposition of fibrinous exudate in alveolar spaces, with moderately diffuse cellular infiltrate consisting predominantly of neutrophils and numerous bacterial colonies (Fig. 1a, arrow). The bronchus showed epithelial hyperplasia with syncytial giant cells formation (Fig. 1b). A morphological diagnosis of acute severe fibrinonecrotic bronchopneumonia was made.

The liver had multifocal widespread areas of vacuolation with focal areas of coagulative necrosis and presence of bacterial colonies (Figure 2). There were perivascular and periportal oedema and cellular infiltration predominantly composed of neutrophils and some macrophages.

The kidney showed severe diffuse tubular degeneration and necrosis with vascular congestion (Figure 3). The spleen showed lymphoid hypoplasia with presence of haemosiderosis and erythrophagocytosis by macrophages.

### *Bacteriological Finding*

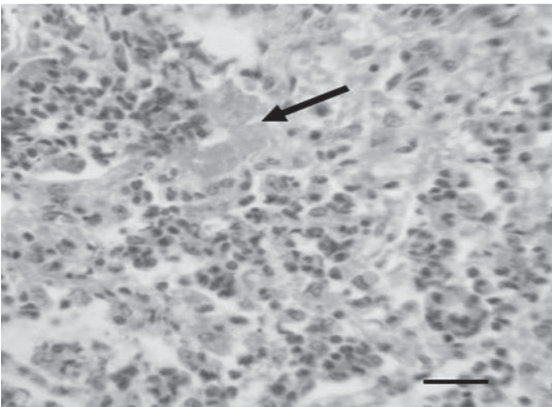
On blood agar plate, the organism appeared as round, greyish, small colonies surrounded by narrow zones of  $\beta$ -haemolysis. On agar plate made with the blood of very young sheep, a double zone of  $\beta$ -haemolysis was

**Table 1:** Cultural and biochemical characteristics of the bacterium isolated from lymph nodes, nasal swab and lung samples of the WAD sheep

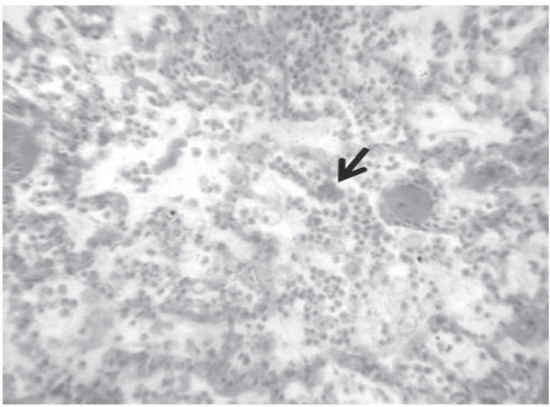
Test	Observation
Oxidase	Positive
Catalase	Positive
Acid/gas from: Glucose	Acid
Sucrose	Acid & gas
Maltose	Acid & gas
Trehalose	Negative
Growth on MacConkey agar	Growth
Motility	Non-motile
Haemolysis on sheep blood agar	Narrow zone of β-haemolysis
Haemolysis on very young sheep blood agar	Double zone of β-haemolysis

**Table 2:** Antimicrobial susceptibility pattern of the bacterial strain isolated from lymph node, nasal swab and lung samples of the WAD sheep.

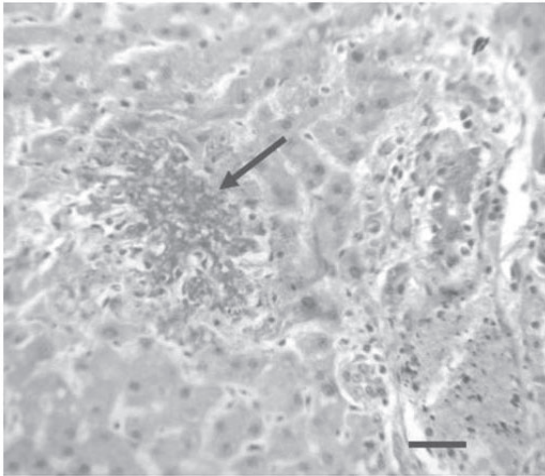
Anti Microbial Agent (µg)	Zone Of Inhibition (mm)	Remark
Furazolidone (50)	24	Sensitive
Ciprofloxacin (10)	26	Sensitive
Norfloxacin (10)	26	Sensitive
Amoxycillin (10)	0	Resistant
Streptomycin (10)	0	Resistant
Gentamycin (10)	0	Resistant
Trimethoprim (50)	0	Resistant
Trimethoprim (50)	0	Resistant



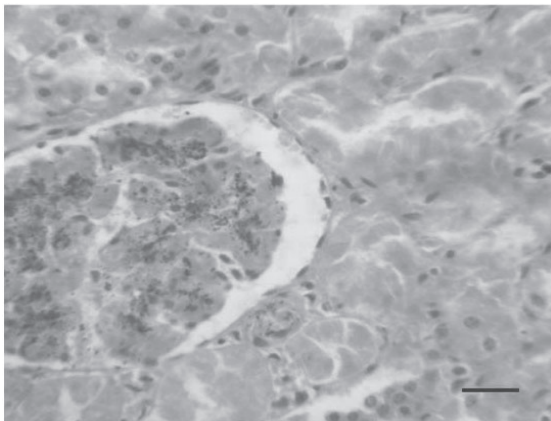
**Figure 1a:** Histological section of the lung showing diffuse severe neutrophilic infiltration with presence of bacterial colony (arrow) (H &E) Bar =10 µm



**Figure 1b:** Histological section of the lung showing syncytial giant cell (arrowed)



**Figure 2:** Histological Section of the liver showing focal areas of necrosis (arrow) with widespread hepatic vacuolation. (H & E) Bar = 50µm



**Figure 3:** Histological section of the kidney showing diffuse severe tubular degeneration and necrosis. (H & E) Bar = 50µm

produced. Smear from the colonies revealed small Gram-- negative coccobacilli, with a bipolar staining appearance. The strain was non-motile, oxidase and catalase -positive but indole- negative. Acid and gas were produced from maltose and sucrose while only acid was produced from glucose. Neither acid nor gas was produced from trehalose by the organism but it grew well on MacConkey agar (Table 1). The pattern of its antimicrobial sensitivity is shown on Table 2. The organism was sensitive to furazolidone (50µg), ciprofloxacin (10µg) and norfloxacin (10µg) using approved standard document M2-A6 (NCCLS, 1997).

## Discussion

This paper describes a case of fulminating respiratory disease associated with *Mannheimia haemolytica* biotype A. *Mannheimia* species which are common commensal of mucous membranes of domestic animals worldwide had been shown to cause pneumonic conditions following a predisposing viral, bacterial or mycoplasma infection (Brogden *et al.*, 1998, Shiferaw *et al.*, 2006) and stress associated with climatic change and/or dexamethasone administration (Zamri-Saad *et al.*, 1991). Primary infection of the lower respiratory tract with virus or bacteria had been reported to increase the susceptibility of sheep and goat to secondary *Mannheimia haemolytica* infection (Brogden *et al.*, 1998). In the present outbreak, the clinical findings, gross and histopathological lesions observed were consistent with *Mannheimia haemolytica* infection in sheep and goats; these findings were in accordance with the report of Zamri- Saad, *et al.*, (1991) and Emikpe and Akpavie (2010). Pneumonia in sheep and goat had been reported to be primarily caused by viral or mycoplasma infection and commonly complicated by *Mannheimia sp* (Ramirez-Romero and Brogden 1995; Brogden *et al.*, 1998, Shiferaw *et al.*, 2006) which was observed in this case. Previous workers (Brogden *et al.*, 1998, Whiteley *et al.*, 1992) reported that small ruminant respiratory diseases often have a multifactorial origin, which may have been the case in this outbreak. Therefore it is logical to suggest that primary infection might have occurred as a result of new infection or a flare-up of existing sub-clinical PPR and this is evidenced by the presence of giant cell pneumonia, observed in the lung tissue histologically. This is suggested to have been stimulated by environmental/climatic change and/or transportation stress coupled with poor management practices. It is note-worthy that the outbreak occurred in February, the peak of the dry season, when the weather is extremely cold in Nigeria.

The primary method of prevention and control of Mannheimiosis in ruminants had been suggested to involve routine mass vaccination (Brogden *et al.*, 1995) and by

using antimicrobials to which the organism is sensitive (Shiferaw *et al.*, 2006) in an infected flock. In the present outbreak, the result of sensitivity test showed that the organism was sensitive to furazolidone (50ug) ciprofloxacin (10µg) and Norfloxacin (10µg). Norfloxacin was selected and promptly used with good result. Interaction of a number of factors had been reported to predispose animals to acute respiratory disease (Shiferaw *et al.*, 2006), therefore, the reduction of stressful conditions as well as provision of supplementary feed, flock immunization against PPR are important elements of preventive herd health programme. Zoonotic potential of *Mannheimia haemolytica* had been reported (Biberstein, 1979, Takeda *et al.*, 2003), hence further investigation on the serotyping and the impact of emerging resistant strains of this organism on human health is required. There is need for the development and standardization of a rapid inexpensive diagnostic test for screening on the field prior to confirmatory laboratory diagnosis as part surveillance and control programme.

### Acknowledgments

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## PROSPECT OF DEVELOPING LOCAL VACCINES AGAINST FOOT-AND-MOUTH DISEASE IN SUDAN

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### Summary

A type "O" antigen of FMD virus derived from a Sudanese isolate (O-Jaz 1/08), and inactivated with binary ethylenimine, produced a satisfactory neutralizing antibody response in 4 inoculated calves. Post-inoculation sera produced high rI values (0.91 and 0.99) with two currently circulating Sudanese type "O" viruses; also indicative of likely protection. The antigen was clarified by simple centrifugation, adjuvated with saponin and aluminium hydroxide gel and 3 ml dose was used to vaccinate animals. The gel was prepared by simple procedure that required no special installation. No adverse effects were observed in inoculated calves following 2-3 doses of the antigen in an elapse of two months; one animal received as high as 12 ml of the antigen.

Homologous neutralizing antibody titres reached a peak of  $2.5 \log_{10}$  two weeks following primary vaccination. Six weeks intervals between the 1st and 2nd dose resulted in a better neutralizing response than that which followed a 4 weeks interval. The best secondary neutralizing response was obtained when 0.5 ml of the antigen was used for priming followed by 3 ml 4 weeks later. It reached a height of  $2.85 \log_{10}$  and remained above  $2 \log_{10}$  up to seventy days post the 2nd dose; last time tested.

**Keywords:** FMD-Inactivated type "O" antigen-'AS' vaccine-Neutralizing antibody response-rI values

## PERSPECTIVE DE MISE AU POINT DE VACCINS CONTRE LA FIÈVRE APHTEUSE AU SUDAN

### Résumé

Un antigène de type « O » du virus de la fièvre aphteuse provenant d'un isolat soudanais (O-Jaz 1/08) et inactivé avec l'éthylèneimine binaire, a produit une réaction satisfaisante des anticorps neutralisants chez 4 veaux inoculés. Les sérums post-inoculation ont produit des valeurs rI élevées (0,91 et 0,99) avec deux virus soudanais de type « O » actuellement en circulation; également évocateurs d'une éventuelle protection. L'antigène a été clarifié par simple centrifugation, de la saponine et du gel d'hydroxyde d'aluminium y ont été adjoints; et une dose de 3 ml a été utilisée pour vacciner les animaux. Le gel a été préparé par une procédure simple ne nécessitant pas d'installation particulière. Aucun effet indésirable n'a été observé chez les veaux inoculés après 2-3 doses de l'antigène sur une période de deux mois; un des animaux a reçu une dose d'antigène aussi élevée que 12 ml.

Les titres d'anticorps neutralisants homologues ont atteint un pic de  $2,5 \log_{10}$ , deux semaines après la primo-vaccination. Des intervalles de six semaines entre la 1ère dose et la 2ème ont abouti à une meilleure réaction de neutralisation par rapport à celle qui a suivi un intervalle de 4 semaines. La meilleure réaction neutralisante secondaire a été obtenue lorsqu'une dose de 0,5 ml de l'antigène a été utilisée pour l'amorçage, suivie d'une dose de 3 ml 4 semaines plus tard. Elle a atteint un pic de  $2,85 \log_{10}$  et est restée supérieure à  $2 \log_{10}$  jusqu'à soixante-dix jours après la 2ème dose, lors de son dernier test.

**Mots-clés:** Fièvre aphteuse – Antigène inactif de type "O" – Vaccin 'AS' – Réponse d'anticorps neutralisants – Valeurs rI

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## Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals. The causative agent is a virus of the family picornaviridea genus aphtoviruses of which seven immunologically distinct serotypes are known. Within each serotype antigenic variation occurs as a continuous process of antigenic drift (Fenner *et al.*, 1987). This antigenic heterogeneity complicates laboratory diagnosis and selection of appropriate vaccines to control outbreaks in the field while, on the other hand, it also facilitates tracing of epizootic spread of FMD viruses (Ferris and Donaldson, 1992).

The disease is a major constrain to efficient animal production in Sudan and elsewhere in Africa. In Sudan clinical disease occurs in cattle only (Abu Alzein, 1983) and recent data indicated the maintained activity of three serotypes "O", "A" and "SAT2" (Raouf *et al.*, 2010). Control of FMD in enzootic areas like Sudan is likely to depend on restriction of animal movement and vaccination. Commercial FMD vaccines of high quality are available. Nonetheless, embarking on vaccination against FMD can be a complicated process. Beside the complex epidemiology of the disease, FMD vaccines themselves are expensive. On the other hand, reliance on commercial vaccines is feared to dismantle elements of national disease control. Such elements like surveillance, early warning, laboratory diagnostic services, vaccine matching and selection studies, monitoring, planning and management of control plan are indispensable for any realistic control efforts of FMD.

In this work, the prospect of developing local vaccines from Sudanese isolates was investigated. The efficacy of the candidate vaccine was evaluated by two major factors; first its immunogenicity in terms of the level of induced neutralizing antibody and secondly its antigenic relation with circulating field viruses. For effective application of the candidate vaccine, vital elements for a successful vaccination campaign like proper inactivation, suitable adjuvant and proposed vaccination regimen were explored.

## Materials and Methods

### *Vaccine virus:*

A local Sudanese isolate of the serotype "O" designated O-Jaz 1/08 (Raouf *et al.*, 2010) was used for production of the antigen. The virus was adapted to grow in cultured cells through 12 passages; 11 in calf kidney cells (CKC) and one in baby hamster kidney cells (BHK). The character of adaptation was defined, as the virus growth that effect absolute determination of titre of infective material. In addition, the seed material used for production of the antigen constantly produced titres between 106.5 and 7.0 TCID<sub>50</sub> and complete cytopathic effects (CPE) in BHK monolayer cultures in about 18 hours.

### *Antigen production:*

Forty-eight hours old BHK21 cell cultures in large Roux bottles were used for production of antigen. Outgrowth medium was Glasgo minimum essential medium (GMEM) containing 0.0487% NaHCO<sub>3</sub> (W/V), supplemented with 10% tryptose phosphate broth (TPB) (V/V) and 10% newborn calf serum (NBCS) (Sigma). Cultures received one wash and inoculated with 10 ml of 1/10 dilution of virus in GMEM devoid of serum. Additional 5 ml of virus diluent was added to each culture bottle to avoid drying of cell sheet. Inoculated cultures were incubated at 37 °C for 1-1 ½ hour with tilting every 15-20 minutes. At the end of adsorption time, 100 ml of GMEM containing 2% NBCS and 10% TPB was added to each bottle without discarding the inoculum. Cultures were returned to the incubator and observed microscopically 18 hours later to detect the production of complete CPE. Harvesting was effected by collecting supernatants into the refrigerator.

### *Inactivation:*

The inactivant was 0.1M (20.5gm l-l) of 2-bromo-ethylamine HBr (BEA) in 0.175 N NaOH. Two cycles of inactivation, each of 24 hours, were carried out at cold room temperature (26 °C) according to the method described by Bahnemann (1990). Virus supernatant was clarified by centrifugation at 2000 rpm for 10 minutes, its volume precisely

determined, brought to 26 °C and pH checked to  $\approx 7.4$ . The solution of BEA was added at the rate of 3% of the virus suspension (V/V) to have a final concentration of 3 mM of binary ethylenimine (BEI); the active substance.

All the process of preparation of the inactivant was carried out in a non-ducted fume hood (AURA 750L-LABCAIRE). The BEA powder was added to 0.175 N NaOH that contained 0.05% (V/V) of sterile 1% aqueous solution of  $\beta$ -naphthol violet (BNV) indicator. The solution was kept in a closed vessel and incubated at 37 °C for 45 minutes. Upon formation of BEI, in about 15 minutes, the color changed from violet to orange. The preparation was allowed another 30 minutes at 37 °C for complete formation of BEI before use.

The inactivant was added to the virus suspension and kept stirring ( $\approx 2.5$  cycle) in a closed vessel in the fume hood. After 24 hours, the mixture was transferred to another vessel and one more cycle of inactivation was carried out by addition of a fresh volume of the inactivant. Inactivation was stopped by addition of 1 M sterile Na-thiosulphate cold solution at a rate of 10% of the whole volume of BEI solution used.

To study the inactivation kinetics samples were taken at 0, 1, 2 and 3 hours of the early inactivation period. Inactivation was stopped immediately in these samples, infectivity titrations carried out and results used to compute the inactivation rate (regression coefficient) according to the formula:

$$r = \frac{\text{Cov.}(x,y)}{s_x s_y}$$

where

$r$  = regression coefficient

$\text{Cov.}(x,y)$  = sample covariance of the 2 variables  
 $s_x, s_y$  = sample standard deviation of the observed values of  $x$  and  $y$ , respectively

The obtained regression line ( $y = a + bx$ ) was used to calculate the endpoint by made up for  $y$  with the minimum inactivation endpoint. The latter is defined as being one log (10) lower than the titre which gives one infectious unit in the total volume under

inactivation (Bahnemann, 1990). It was log 10-3.7 for an inactivated volume of 500 ml of virus suspension. The difference of inactivation endpoint (calculated) to minimum (DIM) has to be positive i.e. the calculated endpoint has to be lower than the minimum endpoint.

After stopping of inactivation, 15 ml of the inactivated antigen (3% of the inactivated antigen lot) was inoculated onto two Roux BHK culture bottle to test innocuity. Cultures were examined for CPE over two blind passages.

#### *Assay of infectivity:*

Performed monolayers of BHK tube cultures were inoculated with log<sub>10</sub> series of virus dilution in GMEM free of serum. Each dilution was inoculated onto 5 tubes and each tube received 0.2 ml of the respective dilution. Following adsorption for one hour at 37 °C; inoculum was discarded; cultures received 3 washes with GMEM; supplied with maintenance media consisting of GMEM, 2% NBCS and 10% TPB then returned to the incubator. Microscopic examination for CPE and medium changes were carried out daily for 5 days. Titres were calculated according to the method of Kärber (1931).

#### *Adjuvation of antigen and vaccine formulation:*

The antigen was adjuvated with 2% aluminium hydroxide gel and saponin.

Aluminium hydroxide gel was prepared using a simple procedure (Barteling, 2002:a) with slight modification. The method depends on dissolving aluminium oxide ( $\text{Al}_2\text{O}_3$ ) in Na OH to obtain aluminium hydroxide then neutralizing the alkali with HCL. In this work aluminium hydroxide was used instead of  $\text{Al}_2\text{O}_3$ . To prepare 100 ml of the gel, 3 gm of sodium hydroxide pellets (Sigma) were dissolved in 10 ml of DDW then added to 2 gm of aluminium hydroxide (Scharlau Chemie SA). The mixture was autoclaved for one hour at 121 °C. After cooling down, the solution was neutralized with 90 ml of 0.83 M HCL. The resulting gel had a pH of approximately 7.5 and stable. It was autoclaved before use.

The ability of the prepared gel to bind the antigen was checked by mixing with the live virus suspension (before inactivation) at 25% and 40% gel concentrations (V/V).

Mixtures were centrifuged at 2000 rpm for 10 minutes and supernatants were simultaneously titrated together with a sample of the live virus suspension in microtitre plates.

According to results obtained, the antigen was adjuvated with 25% of aluminium hydroxide 2% gel (V/V). Saponin (AppliChem) was dissolved in phosphate deficient diluent and added at the rate of 1.66 mg/ml of the final blend.

#### *Animals' inoculation experiments:*

Four calves of local and cross breeds (two each), were screened negative for antibodies against type "O" FMDV by virus neutralization (VN) test.

Animals were placed in a bio-secure facility. Innocuity of the bulk inactivated virus harvest was tested in two calves; one (No. 288) received one ml intradermally at 10 different sites (0.1 ml per each site) and the other (No. 468) received 7.5 ml intramuscularly (5 ml) and subcutaneously (2.5 ml). Animals were observed for clinical and temperature reaction for 10 days and bled at week intervals.

Immunogenicity of the adjuvated antigen was tested in all four animals. Animals No. 288 and 468 which exposed to the non adjuvated antigen were vaccinated with adjuvated antigen after 10 and 30 days, respectively. Schedule and volumes of the 1st and 2nd vaccine doses are shown in Table 1. Animals were bled weekly for antibody assessment.

#### *Antibody assay:*

Pre- and post-inoculation sera were assayed for type "O" antibody using the quantitative VN microtest in BHK-21 cells (OIE, 2008). Virus stock of O-Jaz 1/08 was diluted to contain 100 TCID<sub>50</sub> in 50 µl. Virus diluent was GMEM containing 0.0487% Na HCO<sub>3</sub> (W/V) and 10% TPB (V/V). Two fold serial dilutions of inactivated (30 minutes at 56 °C) sera in volumes of 50 µl were performed in a microtitre plate from row A to row H starting with dilution 1/4 (final dilution of 1/8) and ending with dilution 1/512 (final dilution of 1/1024). Serum diluent was similar to virus diluent but contained in addition 10% tris-buffer (0.05 M). Each serum was diluted in 3 columns and tested in two columns leaving the

third as serum control. Controls contained in addition, in each run of test, virus and cell controls. Fifty µl of virus dilution was pipetted to all wells except those represented sera and cell controls which received in place virus diluent. Plates were sealed with adhesive tape and incubated at room temperature. After one hour each well was seeded with 50 µl of BHK cells suspended in GMEM containing 10% tris-buffer and 10% NBCS. Plates were sealed with adhesive tape and incubated at 37 °C with a source of humidity. Final microscopic reading was done on the third day post seeding or plates stained with 0.1% crystal violet in 10% formol-saline. Homologous antibody titres were calculated according to the method of Kärber (1931). Pre-inoculation sera tested negative when both wells containing the final serum dilution of 1/32 showed CPE.

#### *Antigenic relationship:*

The post-inoculation sera were used to study the antigenic relationship between the vaccine strain and two type "O" local viruses isolated from Khartoum in 2008 (O-Kh 1/08) (Raouf *et al.*, 2010) and in 2010 (O-Kh 2/010) (Anon, 2010). The rI value was derived using the two-dimensional VN assay. The test was carried out according to Booth *et al.*, (1978) and as described recently (Raouf *et al.*, 2010).

## **Results**

#### *Inactivation and adjuvation of the antigen:*

The inactivated antigen failed to produce CPE in inoculated cultures following two blind passages or to elicit localized or generalized clinical reactions in susceptible calves following intradermally, subcutaneous or intramuscular inoculation. The different parameters of the inactivation process are shown in Table (2). The inactivation rate was around one log unit (-0.979). The calculated end point was lower than the minimum end point and the DIM was positive.

The 25% gel was superior to the 40% gel in adsorption of live virus probably because of more optimum pH. A supernatant of the virus preparation with a titre of 106.7 TCID<sub>50</sub> (the antigen before inactivation) showed no titre following adsorption with the 25% gel

**Table 1:** Schedule and volumes of vaccine doses

Animal No.	Interval between the 1st and 2nd vaccine dose	Volume of the 1st vaccine dose*	Volume of the 2nd vaccine dose*
288	6 weeks	3 ml	
2			3 ml
468	4 weeks		
7		0.5 ml	

\*Type "O" antigen adjuvated with 25% of aluminium hydroxide 2% gel (v/v) and saponin at the rate of 1.66 mg/ml

**Table 2:** Parameters of the inactivation process

Infectivity titration during early hours of inactivation	Hour	Titre*
	0	6.7
	1	5.95
	2	4.7
	3	4.2
Inactivation rate		-0.979
The minimum end point		3.7
Calculated end point		3.45
DIM**		0.25

\*  $\log_{10}$  TCID<sub>50</sub>

\*\* Difference of inactivation endpoint to minimum

**Table 3:** rI values between the candidate vaccine and circulating type "O" FMD viruses

Virus	O-Jaz 1/08 post-vaccination serum rI value
O-Jaz 1/08	1
O-Kh 2/010	0.91
O-Kh 1/08	0.99

while CPE were detected at 10-1 dilution following adsorption with the 40% gel.

#### Immunogenicity:

Results presented in Figure. (1) show that non adjuvated antigen was poorly immunogenic while the adjuvated antigen was a good immunogen. As high as 7.5 ml of the non adjuvated antigen produced a transient poor neutralizing antibody response of one  $\log_{10}$  that disappeared completely by the 21st day post inoculation (animal No.468). On the other hand, 3 ml of adjuvated antigen produced a neutralizing response of 101.65 by the 7th day post vaccination, showed a maximum height of around 102.5 one week later and remained fairly high up to 5 or 6 weeks post vaccination (Figure. 2).

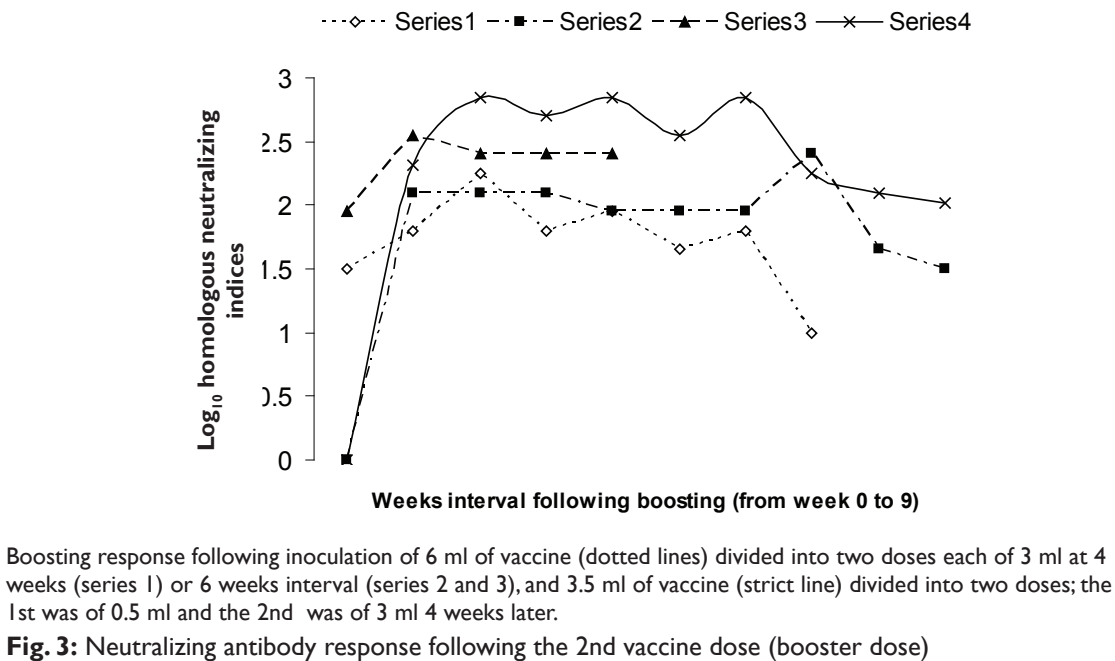
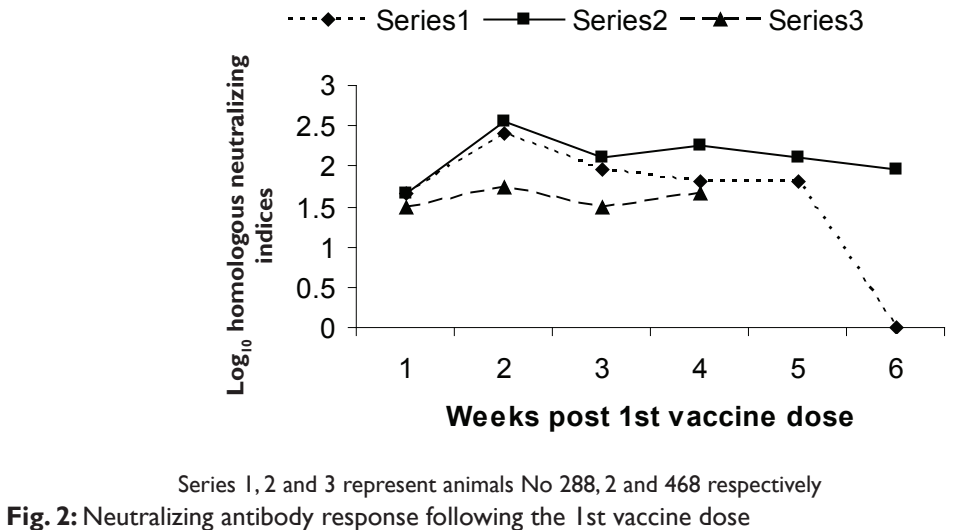
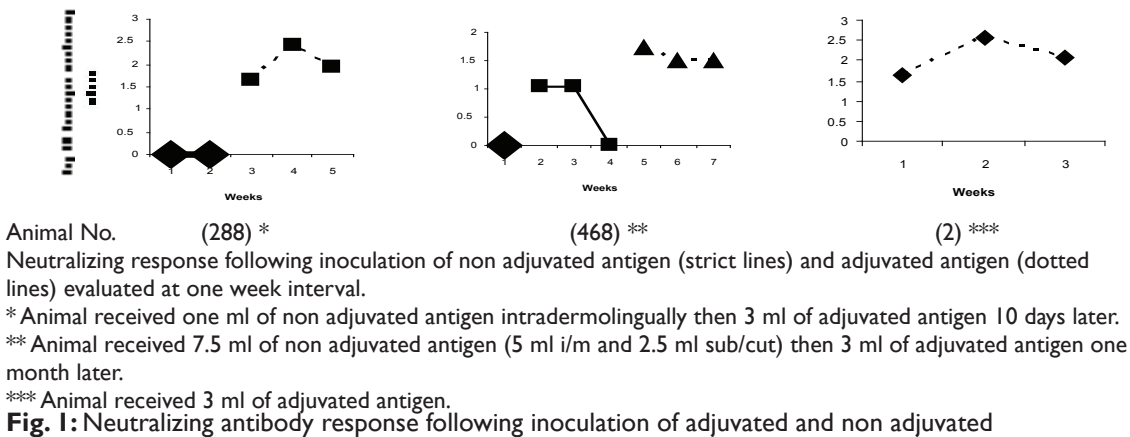
In the two occasions in which the interval between the first and booster dose (each of 3 ml) was 6 weeks, it showed better anamnestic response in term of intensity and duration than when it was 4 weeks (Fig. 3). The best anamnestic response was obtained when 0.5 ml of the antigen was used as a first dose followed by a booster dose of 3 ml 4 weeks later (Figure. 3). The 0.5 ml dose produced no measurable neutralizing response but the booster response showed the highest peak (2.85  $\log_{10}$ ) in this work; higher by 0.3 log than the nearest peak (i.e. double intensity) and remained above 2  $\log_{10}$  up to 70 days post-boostering (last time tested).

#### Likely heterologous protection:

The post vaccinal sera produced high rI value, above 0.9 (Table 3), with two circulating type "O" viruses (O-Kh 1/08 and O-Kh 2/010) isolated in 2008 and 2010 in Khartoum State.

## Discussion

The data presented in this work show that an antigen produced from a local Sudanese isolate of type "O" FMD virus elicited a satisfactory neutralizing antibody response in



inoculated calves. The antigen was produced as a crude bulk virus harvest, clarified by simple centrifugation and delivered as an 'AS' vaccine. No adverse effects were observed in inoculated calves, neither following vaccination nor following inoculation of high doses of the inactivated antigen. Antigen payload per dose was not determined and a 3 ml dose was used to vaccinate animals. Six weeks intervals between the 1st and 2nd dose of the vaccine was following by better neutralizing response than that followed 4 weeks interval. Among the significant findings in this work was that priming with 0.5 ml of vaccine instead of 3 ml resulted in the strongest anamnestic response throughout this work. When the post-vaccinal serum was used to derive the rI value with two circulating type "O" FMDV isolated in 2008 and 2010, a high value significant of likely protection was obtained.

A number of studies, using different assays like complement fixation, neutralization and ELISA, have demonstrated close relationship between FMD vaccines induced specific antibodies response and protection against challenge infection (Sutmöller and Vieira, 1980; Pay and Hingley, 1986, 1987, 1992; Hamblin *et al.*, 1986; McCullough *et al.*, 1992; Van Maanen and Terpstra, 1989; Van Maanen, 1990). Furthermore, Sutmöller and Vieira (1980) after examining 791 sera from cattle vaccinated against serotypes "O", "A" and "C", associated neutralization titres above  $1/64$  ( $1.8 \log_{10}$ ) with high level of protection and identified neutralizing titres between  $1/8$  and  $1/32$  ( $0.9$  to  $1.5 \log_{10}$ ) as difficult to interpret in terms of protection at challenge. McCullough *et al.*, (1992), explained that although the correlation between different assays and protection could not be precise, they described 3 zones of neutralizing antibody titre: above 101.3 where animals are likely to be protected, below 100.7 where animals are likely to be susceptible and an in between zone (from 0.7 to  $1.3 \log_{10}$ ) where no prediction could be made. In this work, response obtained 7 days following inoculation of a 3 ml dose and thereafter (Figure 2 and 3) up to 70 days following the 2nd dose was well above  $1.5 \log_{10}$  neutralization titre. In 50 trials to measure the neutralizing response throughout the testing period 40 times the neutralization titres were

above 101.8, 6 times between 101.5 and 101.8 and only in four occasions it was below 101.5.

It was feared before this work that more than one local variant of type "O" is required to be incorporated in local vaccines to protect against field viruses. A high herd immunity above 80% which is indicative of herd protection (Doel, 2003) was detected for types "O" and "A" in Sudan using the Liquid-Phase Blocking ELISA (LPBE) (Raouf *et al.*, 2011), yet, unlike type "A", type "O" clinical disease keeps to reappear frequently (Habiela *et al.*, 2010; Raouf *et al.*, 2010; Anon, 2010). However, in this work as in previous occasions (Abu Elzien and Newman, 1980) high rI value indicative of cross protection between circulating type "O" field viruses were detected. A plausible explanation for the described situation is that ELISA measure a wide spectrum of antibodies, a proportion of it may not be related to antibody mediated protective mechanism (Doel, 2003). On the other hand, McCullough *et al.*, (1992) were of the opinion that detection of specific antibody alone with assays such as complement fixation, neutralization and ELISA are incomplete as a measure of the protective immune response. When, Sutmöller and Vieira (1980) examined with neutralization assay about 800 cattle sera following vaccination with types "O", "A" and "C", it was evident that similar neutralization titres were associated with lower protection rates against homologous challenge for type "O" in comparison to type "A" and "C". Similar observations led Pay and Hingley (1987) to make the conclusion that antibodies induced by O1 vaccines appear to be qualitatively inferior to those induced by "C" and "A" viruses. However, since the described field condition in Sudan was observed following natural infection and repeated exposure to type "O" viruses in absence of remarkable antigenic differences, it is justifiable to conclude that the effector protective immune response that follow different serotypes of FMD virus infection or vaccination, e.g. "O" and "A", differ in quality and efficiency. It seems that neither the systems used for monitoring the protective immune response (the LPBE in Sudan) nor serotype "O" vaccines (O1) were responsible for the described situation. Perhaps such deduced qualitative differences in the immune response

and ensuing protection against type "O" share account in its world-wide distribution.

Foot-and-mouth disease antigens are poor immunogens. Adjuvants are required to potentiate immunity. The immunopotential effect of the adjuvant used in this work was shown in (Figure. 1). The immunopotential effect of Al (OH)<sub>3</sub>-gel particularly targeting which is defined as ability of the adjuvant to deliver an immunogen to immune effector cells, generally via antigen presenting cells (APCs), depends on bound of the immunogen to the gel (Cox and Alan, 1997) which, in turn, depends on protein content, pH and ion concentration of the antigen preparation. The antigen preparation in this work was not purified and used as a crude bulk virus harvest. It was essential to verify the optimum condition for adsorption though the used concentration of gel (25%) is the one that generally applied in FMD vaccines. Live virus suspension with a titre of  $6.7 \log_{10}$  TCID<sub>50</sub> were found to be free of virus following simple centrifugation after addition of 25% gel. Targeting as a function of the adsorption capacity of the gel is essential for reducing the amount of immunogen required to achieve a given effect (Cox and Alan, 1997). Aluminium hydroxide-gel/saponin adjuvanted FMD vaccines (aqueous vaccines) have been used successfully in Europe (Fish *et al.*, 1969; Remond *et al.*, 1998; Doel, 1999) while good results were obtained with oil-adjuvanted vaccines in South America (Casas Olascoaga, 1978; Dora *et al.*, 1984; Bahnenmann and Mesquita, 1987; Casas Olascoaga *et al.*, 1999). Though oil-adjuvanted vaccines could prove advantageous in respect to duration of immunity and vaccination of neonates and pigs, yet, without generalization, reported localized reactions following sub/cut inoculation (Barnett *et al.*, 1996) might discourage vaccination programs where clinical signs of FMD are mild and its economic impact is not fully appreciated. Moreover, reports that described persistent carcass reactions following application of oil-vaccines (Garland, 1999) should be carefully regarded whereas millions of doses of aqueous vaccines were used in Europe without raising public health concern.

When innocuity of FMD vaccines is dealt with, two aspects are worthy of

particular consideration. The first is that residual infectivity in FMD vaccines is highly unacceptable and the second is the reported adverse reactions to some FMD vaccines. Non-infectivity can be tested by inoculation of material from the inactivated bulk virus harvest onto sensitive cell culture (OIE, 2008) and, ultimately, according to the European Pharmacopeia (2008) intradermolingually in susceptible cattle. The problem with these tests according to Barteling (2002b) is that they can fail to detect residual infectivity in large volumes of bulk virus harvest and/or their statistical validity be compromised. Inactivated FMD antigens are usually produced in large volumes for example tens or hundreds of litres. It follows that, appropriate in-process control of the inactivation process and verification of the inactivation kinetics are of paramount significance in assessment of FMD vaccine non-infectivity (Barteling, 2002b; Bahenmann, 1990). Accordingly, of particular importance had been the establishment of these procedures in the course of this work. Moreover, since the produced antigen in this case was of small volume, confirmation of innocuity by inoculation of sensitive cell culture and susceptible cattle clearly substantiate safety of future similarly in-process controlled inactivated products.

Adverse allergic reactions to FMD vaccines has been reported at low rates; lower than 0.1% in Germany (Lorenz and Straub, 1971) and about 0.27% in Russia (Chepurkin *et al.*, 1975). Nonetheless, surprising increases have been recorded in certain occasions (Black and Pay, 1975); as high as 12.5% (Yeruham *et al.*, 2001). These skin allergic reactions were either delayed (type III) (Kaaden *et al.*, 1971; Bahenmann *et al.*, 1971) or immediate hypersensitivity (type I) (Ubertini and Barei, 1970; Beadle and Pay, 1975) and were associated with repeated vaccination (Black and Pay, 1975; Yeruham *et al.*, 2001) while extent of lesions and high rates were more linked to concurrent infections such as bovine viral diarrhoea-mucosal disease complex and Johne's disease (Yeruham *et al.*, 2001). The responsible allergen has not been identified beyond doubt and equally none of the major vaccine components has been conclusively clarified. Inoculated animals in this work showed no adverse effect; all were

inoculated twice or thrice over a period of two months. One animal received as high as 12 ml of the crude bulk harvest, yet it showed no untoward effect.

It is common practice to vaccinate cattle, particularly young animals, with two doses of FMD vaccine separated by an interval of 3-4 weeks followed by regular vaccination every 4-6 months. The longer the interval between the first and the second booster dose the stronger the anamnestic response (Doel, 1999 and 2003). Results obtained in this work were similar to these findings. Exposure to the antigen 4 weeks earlier (calf No. 468) result in the lowest neutralizing response following vaccination (figure. 2 and 3). The two animals that received the booster dose 6 weeks following the first dose showed a better secondary response (figure 3), irrespective of their neutralizing antibody levels at the time of boosting. Four weeks interval results in an intermediate response, and largely recommended because it is suitable to most epidemiological situation (Doel, 1999). Following boosting at week 4, SAT1 and 2 antigens, delivered as an AS vaccine beside SAT3 antigen, failed to produce an anamnestic response at all (Cloete *et al.*, 2008). In this work, animal No. 468 showed an anamnestic response following each exposure, in the sense that it was markedly higher than the previous one, but it remained lower in intensity and duration than in other cases. The strongest anamnestic response in this work was obtained when the animal was primed with 0.5 ml of vaccine in the first dose followed by a 3 ml dose 4 weeks later. Such animal showed no neutralizing response following the first dose, but a strong one following the second dose that reached about  $3 \log_{10}$  in magnitude and remained above  $2 \log_{10}$  up to 10 weeks post the second dose. Similar to these findings, Black *et al.*, (1984) reported that the anamnestic response was most marked when low-antigen doses were used initially. Doel (1996) commented on these findings that two products could be developed; the first to prime the immune system for optimum performance on revaccination and the second to boost the immune response. It is recommended that annual vaccination against FMD should be carried out in October and

November before the cold season (Barteling *et al.*, 2004) which is suitable for epidemiological situation in Sudan and other enzootic areas in Africa. The chance for stronger anamnestic response in young animals by using low-antigen doses in the primary vaccination should not be neglected but thoroughly investigated. In addition, reducing the volume of the primary vaccine dose by 4 or 6 fold imply effective reduction in the cost of FMD vaccine production.

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### Impact

Foot-and-mouth disease vaccines are expensive. Whereas, 3 ml of the final vaccine blend could be used to vaccinate 400 animals in rinderpest vaccine, it would be used to vaccinate one animal in case of FMD vaccines. In addition, two doses are required for primary vaccination and vaccination is to be repeated every 4-6 months. Commercial FMD vaccines of high quality are available. The presented work proposes production of local FMD vaccines to reduce cost and to potentiate elements of national disease control programs. The crude bulk virus harvest was used as a vaccine which is a known common practice in FMD vaccines. Further, it applied simple procedure, which required no special installation, for preparation of aluminium hydroxide gel and demonstrated its immunopotential effect. It indicated a lower dose volume for primary vaccination which could reduce cost of antigen production by 4 to 6 folds and at the same time result in a better immune response.

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## RISK FACTORS THAT INFLUENCE THE DISTRIBUTION AND ACARICIDE SUSCEPTIBILITY OF IXODID TICKS INFESTING CATTLE IN RWANDA

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### Abstract

Information about acaricide classes to which ticks are tolerant or resistant to in Rwanda is mainly based on farmer reports of reduced susceptibility and scientific evidence is lacking. This study was designed with the aim of providing in-depth assessment of risk factors basing on acaricide use patterns and their association with distribution of ticks among cattle keepers in the Eastern and Western Provinces. A total of 976 cattle were searched for ticks by whole body search technique of which 205 were infested and 771 were not. Relative abundance of ticks significantly differed by province ( $\chi^2 = 198$ ,  $df = 1$ ,  $P < 0.05$ ) and by district ( $\chi^2 = 271$ ,  $df = 3$ ,  $P < 0.05$ ); of the 205 cattle infested by ticks, 175 (85%) were from the Eastern Province compared with only 30 (15%) cattle from the Western Province. Speciation of ticks showed only three species: *Rhipicephalus appendiculatus* (95.4%), *Boophilus decoloratus* (2.8%) and *Amblyoma variegatum* (1.6%). Genus *Rhipicephalus* was more abundant in the Eastern Province and *Amblyoma* in the Western Province; *Boophilus* was identified only in the Western Province. Acaricide type, frequency of use, area altitude and wash volume among other risk factors for acaricide tolerance / resistance in ixodid ticks are discussed.

**Key words:** Acaricide resistance, acaricide class, tick species, cattle

## FACTEURS DE RISQUE QUI INFLUENCENT LA REPARTITION ET LA SENSIBILITE AUX ACARICIDES DES TIQUES IXODES INFESTANT LES BOVINS AU RWANDA

### Résumé

Les informations sur les classes d'acaricides auxquels les tiques sont tolérantes ou résistantes au Rwanda sont principalement fondées sur les rapports des éleveurs faisant état d'une sensibilité réduite, mais les preuves scientifiques font défaut à ce sujet. La présente étude a été conçue dans le but de fournir une évaluation approfondie des facteurs de risque sur base des modes d'utilisation et de l'association des acaricides avec la répartition des tiques chez les bovins dans les provinces de l'Est et de l'Ouest. Au total, 976 bovins ont été examinés en vue de rechercher la présence de tiques, la méthode utilisée consistant à explorer tout le corps de l'animal. Cette méthode a révélé que 205 étaient infestés et que 771 ne l'étaient pas. L'abondance relative des tiques différait significativement selon la province ( $\chi^2 = 198$ ,  $df = 1$ ,  $P < 0,05$ ) et le district ( $\chi^2 = 271$ ,  $df = 3$ ,  $P < 0,05$ ). En effet, des 205 bovins infestés par les tiques, 175 (85%) provenaient de la province de l'Est, par rapport à 30 bovins seulement (15%) de la province de l'Ouest. La spéciation des tiques a révélé uniquement trois espèces : *Rhipicephalus appendiculatus* (95,4%), *Boophilus decoloratus* (2,8%) et *Amblyoma variegatum* (1,6%). Le genre *Rhipicephalus* était plus abondant dans la province de l'Est et *Amblyoma* dans la province de l'Ouest ; *Boophilus* a été identifié uniquement dans la province de l'Ouest. Le type d'acaricides, la fréquence de leur utilisation, l'altitude de la zone et le volume de lavage, entre autres facteurs de risque pour la tolérance / résistance aux acaricides chez les tiques ixodes, sont abordés.

**Mots-clés:** résistance aux acaricides ; classe des acaricides ; espèces de tiques ; bovins

## Introduction

Ticks are obligate blood-feeding ectoparasites of animals; they belong to class Arachnida and Order Acarina. The other acarines of veterinary importance are mites. The ixodids are important vectors of protozoan, bacterial, viral and rickettsial diseases. Once ticks attach to their hosts for a blood meal, they can cause diverse effects including blood loss, tick worry, damage to skins, and introduction of toxins (Morel, 1989). There are over 889 species of ticks in the world that are of human and veterinary importance (Tamiru and Abebaw, 2010) and over 79 species in eastern Africa, but many of these appear to be of little significance. It is estimated that 80% of the world's cattle are infested with ticks (Minjauw and McLeod, 2003). The annual global cost associated with ticks and tick borne diseases in cattle range from 13.9 to 18.7 billion dollars (De Castro, 1997).

Tick-borne diseases are a constraint to livestock production in many developing countries as they cause high morbidity and mortality, which results in decreased production of meat, milk and other livestock by-products. The most important tick-borne diseases of livestock in sub-Saharan Africa are East Coast Fever (caused by *Theileria parva*), Babesiosis (caused by *Babesia bigemina* and *B. bovis*), *Anaplasmosis* (caused by *Anaplasma marginale*) and Heartwater (caused by *Ehrlichia ruminantium*). In Rwanda, East Coast Fever (ECF) was documented as the main tick-borne disease of cattle although *Anaplasmosis*, *Babesiosis* and *Cowdriosis* were thought to be of secondary importance (Bazarusanga, 1999). Despite their economic importance, information on the epidemiology of these diseases in many countries, including Rwanda, is often inadequate, making rational disease control strategies difficult to implement.

The distribution of different species of ticks may be attributed to livestock management system, host density, vegetation cover and other ecological factors (Salih *et al.*, 2004). High altitude and relatively low annual average temperature may play an important role regarding specific tick species abundance. The 21<sup>st</sup> Century is facing global climate change

that could pave way for invasion of tick species hitherto unknown in certain locations. For instance, it is envisaged that the Mediterranean region will have suitable climate for survival of ticks from regions like the Middle East (Gay *et al.*, 2008). Rwanda being a country of clear varied agro ecological zones defined by altitude and mean temperature, this would imply that these factors have an effect on the distribution of ticks and consequently tick borne diseases. Therefore, successful control of ticks and Trans Boundary Disease can only be achieved by increasing understanding of the distribution and dynamics of ticks and the control methods presently in use.

Reports from the eastern parts of Rwanda show an increase in cases of reduced susceptibility of ticks to currently used acaricides (Personal Communication). The situation in other parts of the country is not yet established. Although a limited number of chemical classes is recommended for use by the Directorate of Animal Resources, the actual drugs in use in the field need to be ascertained. This study, therefore, aimed at generating baseline data about the major species of ticks infesting cattle and the chemicals in use to control ticks as well as application methods.

## Materials and Methods

### The study area

The study was conducted between June and July 2011 in the Eastern and Western Provinces of Rwanda. From each province, 2 districts were selected and from each district, 2 sectors were selected. The districts and sectors for study were chosen by assigning random numbers generated by Excel 2007 {=rand( )} function and picking those with the highest probability scores. In the Eastern Province, Nyagatare district (Musheri and Nyagatare Sectors); Rwamagana District (Muhazi and Nyakariro Sectors) were selected. In the Western Province, Nyamasheke district (Bushenge and Kagano Sectors); Rutsiro district (Mushubati and Manihira Sectors) were selected. The two provinces differ in altitude; the Eastern province is largely plateau while the Western Province is mainly highland.

### Questionnaire administration

A structured questionnaire was administered to farmers by interview. Specific questions targeting acaricide chemical class by trade name, spray frequency, whether ticks died after application of acaricide were asked and recorded. The data were entered in Excel 2007 spreadsheet; analysis was done with SPSS v. 16; correlations, frequencies and Chi Square tests were used to determine level of significance between variables.

### Study animals and sampling methods

The study animals included Ankole Long Horn (ALH), various crossbreeds of ALH with Friesian, Jersey, Sahiwal and Brown Swiss. A total of 976 cattle were sampled and belonged to farmers who participated in the ticks' control interviews. At any farm, a maximum of 10% of animals were selected depending on the herd size.

### Collection of ticks and identification

Whole body search was utilised to collect ticks from cattle; ticks from an individual animal were carefully picked by hand and put in separate Bijour bottles containing 70% ethanol. All body regions were carefully searched for ticks and particular attention was paid to the tail switch, udder / scrotum, under the belly, withers, sacrum, base of horn and ears. All the collected ticks were taken to the Rwanda Central Veterinary Laboratory, Kigali, division of Parasitology for identification according to a guide by Walker *et al.*, (2003). Female ticks were differentiated from males by presence of a scutum covering most of the dorsal surface.

### Classification of ticks according to feeding status

The feeding status of female ticks was classified as engorged if a tick had taken a full blood meal; 'fed' if it was not fully engorged and 'not fed' if she was newly moulted and had not started engorging.

### Statistics

Frequencies of numbers of ticks, their sex, species and stage of development; the frequency tables for acaricide use by farmers, frequency of application, volume applied per animal were done in Excel 2007. Chi-square

tests were used to analyse the relative abundance of ticks by region and district in SPSS v 16.

## Results

### Species diversity of ticks infesting cattle in Rwanda

Ixodid ticks infesting cattle in two of the four provinces in Rwanda are shown in Table I.

Genus *Rhipicephalus* was the most picket tick from cattle (912/955, 95%) while *Boophilus* was the second most abundant tick (27/955, 3%) and *Amblyoma* the least (15/955, 1.5%). This implies that East Coast Fever, transmitted by *Rhipicephalus* is the predominant tick borne disease in cattle in Rwanda. Notably, *Boophilus* was not identified in the ticks from the Eastern Province, implying that this vector which transmits Anaplasmosis is of more importance in the Western Province. Relative abundance of ticks significantly differed by province ( $\chi^2 = 198$ ,  $df = 1$ ,  $P < 0.05$ ) and by district ( $\chi^2 = 271$ ,  $df = 3$ ,  $P < 0.05$ ).

### Stage of development and sex of ticks

The ticks were further classified by stage of development and sex; the stage was classified as either adult, nymph or larva. In this study, only adults and nymphs were found in the parasitic stage and no larvae were found.

The stage of development of ticks and sex of individual ticks is shown in Table 2. There were more male than female ticks with a Female: Male ratio of 0.65. This may be due to the fact that females detach from the host once they have engorged to lay eggs on the ground. Nymphs cannot be assigned sex because both sexes are indistinguishable at this stage of development.

### Feeding status of ticks

Table 3 shows the feeding status of ticks at different stages of development. Only female ticks and nymphs were classified according to feeding status; of the 48 engorged ticks, 47 (98%) were *Rhipicephalus appendiculatus* and only one *Amblyoma variegatum*. The time ticks take on their hosts is an important indicator in acaricide efficiency because adult ticks fully engorge in 5-20 days while larvae engorge in

**Table 1:** Ixodid ticks that parasitize cattle in Rwanda: relative abundance by province and district

Species of tick	Eastern Province		Western Province		Total
	District				
	Nyagatare	Rwamagana	Nyamasheke	Rutsiro	
<i>Rhipicephalus appendiculatus</i>	673	197	3	39	912
<i>Amblyoma variegatum</i>	3	8	3	1	15
<i>Boophilus decoloratus</i>	0	0	15	12	27
Unclassified	1	0	0	0	1
<b>Total</b>	<b>677</b>	<b>205</b>	<b>21</b>	<b>52</b>	<b>955</b>

**Table 2:** Stage of development and sex of ticks

Development stage	Sex of ticks				
	Female	Male	Not applicable	Total	F:M ratio
Adult	349	536	2	887	0.65
Nymph	0	0	78	78	
Larvae	0	0	0	965	

**Table 3:** Feeding status of ixodid ticks

	Engorged	Fed	Not fed	Total
<i>Rhipicephalus appendiculatus</i>	47	104	192	344
<i>Boophilus decoloratus</i>	0	0	1	1
<i>Amblyoma variegatum</i>	1	3	1	5
<b>Total</b>	<b>48</b>	<b>107</b>	<b>194</b>	<b>349</b>

3-5 days (Walker et al., 2003).

Of 349 female and nymphs combined, only 48 (14%) were engorged implying longer time of attachment onto hosts at least above 7 days ; 107/349, 31% were fed (moderate time spent on the host) of between 3 and 5 days and 194/349, 56% were newly hatched and not sucked a blood meal (less than 2 days).

*Acaricide spectrum in the study area*

The acaricides by generic names used in the study area classified according to their chemical classes and are summarized in Table 4.

The acaricides in use were in four categories but under various trade names. These are Avermectins, Dinirophenyl compounds, Amitrazes, and Synthetic pyrethroids. One farmer used acaricide that was already constituted and did not know the

trade name; the majority of farmers mainly from the Western province did not spray their cattle (205/497, 41%).

*Frequency of spray at standard concentration*

The frequency of spraying at standard concentration is shown in Table 6 while the volume of acaricide at standard concentration as a risk factor to acaricide resistance to ticks is presented in Table 5.

The reconstituted acaricide wash measured in litres is shown in Table 4; 45% of the farmers spray with 2-3 litres per animal on a spraying day. The category ‘not applicable’ were farmers that did not spray and the ‘not sure’ category were those who could not recall how much reconstituted acaricide they sprayed with.

**Table 4:** Classification of acarides in use in the Eastern and Western Provinces

Trade name	Chemical class	Frequency of use
Cypermethrin 30%	Cypermethrin (Synthetic pyrethroid)	177
Renegade	Alphacypermethrin (Synthetic pyrethroid)	2
Protaid	Synthetic pyrethroid	1
Decatix	Deltamethrin (Synthetic pyrethroid)	6
Deltanex	Deltamethrin (Synthetic pyrethroid)	1
Bayticol	Flumethrin (Synthetic pyrethroid)	51
Acaramik	Avermectin	1
Dinitro	Dinitrophenyl	6
Amitraz	Amitraz	2
Milbitraz	Amitraz	44
Premixture	Not known	1
Do not spray	Not applicable	205
<b>Total</b>		<b>497</b>

**Table 5:** The volume of reconstituted acaricide spray used in cattle

Volume of acaricide wash	Frequency	Percent
< 1 litre	26	5
2-3 litres	225	45
4-5 litres	11	2
Not sure	33	7
Not applicable	202	41
<b>Total</b>	<b>497</b>	<b>100</b>

*Spray frequency by province*

The spray frequency of acaricide in weeks in Eastern and Western provinces is shown in Table 6.

The majority of farmers sprayed twice a week (165/498, 33 %) followed by spraying once a week (126/498, 25 %). Those designated 'na' (not applicable) did not spray at all and a few of those had not started spraying because they said they had just received the animals. Equal numbers in eastern and western provinces sprayed once a month (4/498, 0.8 %).

*Indicators of acaricide resistance*

Farmers were asked when they sprayed at standard concentration whether all ticks were killed. The responses are summarized in Table 7. The responses indicate increased tolerance of ticks to currently used acaricides and hence possible resistance.

After spraying with the acaricide at standard concentration, some farmers in

the Eastern Province reported that not all ticks were killed (22/497, 4 %). None of the farmers in the Western province reported that observation. However, the majority of farmers in the Western Province did not find need to spray the animals because they reported they had not encountered ticks on their animals.

**Discussion**

According to results from this study, the relative abundance of the parasitic stages of ixodid ticks differed by province ( $\chi^2 = 198$ ,  $df = 1$ ,  $P < 0.05$ ) and by district ( $\chi^2 = 271$ ,  $df = 3$ ,  $P < 0.05$ ). The differences can be explained by climatic factors that have been suggested as determinant in tick infestation rates (Siddig *et al.*, 2010); Cadenas and Burri, (2008) showed that tick densities decreased at higher altitudes. This is in agreement with findings from the present study because the tick numbers picked from the western high plateau were significantly

**Table 6:** Spraying frequency by province

Frequency of spraying	Province		Total number of farmers
	# Eastern	# Western	
Once a week	91	37	128
Twice a week	141	25	166
Three times a week	6	4	10
10	10	8	18
Once a month	4	4	8
Do not spray	15	152	167
<b>Total</b>			<b>497</b>

**Table 7:** Standard concentration use of acaricide and tolerance by ticks

All ticks killed after spray					
Province	District	#Yes	# No	Not applicable	Total
Eastern	Nyagatare	107	13	1	121
	Rwamagana	123	9	12	144
Western	Nyamasheke	34	0	60	94
	Rutsiro	41	0	97	138
<b>Total</b>		<b>305</b>	<b>22</b>	<b>170</b>	<b>497</b>

lower than numbers from the eastern plateau. The other major factor on occurrence and tick infestations on animals is seasonality. Rwanda is a country divided into 4 major agro climatic / ecological zones. This study was done in two of the four zones, namely western high plateau at average 1,150-1,900 metres above sea level, average annual rainfall of 1,100-1,250 mm and average annual temperature between 19-21°C. Comparatively, the other area of study was the Eastern Plateau, average height above level of 1,000-1,550 metres, average annual temperature of 21-24°C and lower average annual rainfall of 800-900 mm.

There are limited studies on the distribution of ticks by species in Rwanda; ticks recovered from cattle in the present study belonged to three genera and species, namely *Rhipicephalus appendiculatus*, *Boophilus decoloratus* and *Amblyoma variegatum*. In an earlier study, Bazarusanga et al., (2007) found three species and six genera (*R. apppendiculatus*, 91.8%; *B. decoloratus*, 6.1% and *A. variegatum*, 1.2%). Few ticks from the other less economically important species were also recovered viz. *R. compositus*, *R. evertsi* *evertsi* and *Ixodes cavipalpus*. Nshimiyimana and Mutandwa (2010) found four genera and

five species; *R. appendiculatus* was the most prevalent tick species and composed 96% of the tick species recovered. These findings are in agreement with those of the present study where *R. appendiculatus* accounted for 95% of the recovered ticks from cattle. The relative abundance of the ticks seems not to have changed in the last two decades: Lessard et al., (1990) reported that *R. appendiculatus* was present in 96% of Rwanda territory. In a follow-up study (results not reported in this paper), the less important *R. evertsi* was recovered from cattle on a farm in the Eastern Province. *Rhipicephalus appendiculatus* is the major vector of *Theileria parva*, the causative agent for East Coast Fever (ECF) and it can be concluded that ECF is the major tick-borne disease afflicting cattle in Rwanda. The disease is of more economic importance in the eastern region because in the present study, 870 of 912 (95%) *R. appendiculatus* ticks were from the eastern region.

Genus *Boophilus* was not recovered from cattle in the Eastern Province although it this species was recovered from cattle reared in the Western Province. This may be attributed to full susceptibility of *Boophilus* to currently used acaricides in this region or actually,

*Boophilus* may not survive in this region. In the tropics, *Boophilus* species is the main vector of anaplasmosis in cattle while *Dermacentor* species is the main vector in United States (Merck Veterinary Manual, 1997). This implies that Anaplasmosis may not be as important in this region compared to ECF that is transmitted by *R. appendiculatus*. However, *Anaplasmosis* may still occur in this region because other less efficient vectors include *R. appendiculatus*. *Anaplasmosis* is more important in the Western region as responses from sector and district veterinarians showed. *Boophilus* is a one host tick and campaigns of intense utilization of acaricides are more effective in eliminating this species compared to the 3-host *Rhipicephalus* where non-parasitic stages of the tick are not found on the animal and certainly escape acaricide contact when applied on the animal. *Amblyoma variegatum* was found in both provinces implying that *Cowdriosis* may equally be of importance in both regions.

The feeding status of female ticks and larvae can be utilized to make inference to the number of days a tick has been attached to the host: ticks feed up to 14 days to be fully engorged and thereafter drop from the host. All the ticks in this study were picked from the hosts implying they were not fully engorged. The majority of engorged ticks were attached to hosts for a period ranging from 7 to 14 days. The assumptions to be made here are two: i) farmers spray regularly and hence these ticks are not susceptible to the utilized acaricides or ii) acaricide was not properly applied and did not reach the target sites where ticks attached. In the eastern region, the majority of ticks that were recovered were *R. appendiculatus* (97%) and *A. variegatum* (3%), implying the phenomenon of reduced acaricide susceptibility / resistance is more important in *R. appendiculatus* than other common tick species in Rwanda.

The chemical class of acaricides, volume of constituted and applied to the animal and frequency of application are among the risk factors for acaricide resistance that were investigated in the present study. Overall, the acaricides used in the study area were broadly classified in four categories and these are i) Amitrazes (Milbitraz, Amitraz); ii) Avermectins

(Acaramic); iii) Dinitrophenol compounds (Dinitro); iv) Synthetic pyrethroids, (Protaid); Flumethrin (Bayticol); Deltamethrin (Decatix and Deltanex). Cypermethrin, Bayticol and Milbitraz were the most used acaricides by 36%, 10% and 9% of the surveyed farmers respectively. Therefore, acaricide resistance in Rwanda is more likely to develop against the three compounds than the other classes which are less in use. Acaricide resistance is defined as reduced susceptibility of ticks to acaricides when used at recommended concentrations. Results from the present study show that all the farmers that have increased concentration of acaricide to kill ticks were all from the Eastern Province and none from the Western province; in the Eastern Province, the majority were from Nyagatare district. Therefore, efforts to investigate the phenomenon of acaricide resistance should be investigated with emphasis on Nyagatare district. However, there are temporal factors to development and distribution of acaricide resistance (Foil et al., 2004) and therefore, need to monitor different acaricides for resistance in farms of interest at different times.

Acaricide resistance is an inherited phenomenon, resulting from exposure of populations of ticks to acaricides and survival and multiplication of ticks that are less susceptible. Effective management of resistance of ticks to acaricides largely depends on understanding the underlying mechanisms as well as risk factors associated with resistance. Risk factors include intensity of application of acaricides (Van Leeuwen et al., 2010) and resistance is also influenced by genetics, biology / ecology and control operations (Georghiou and Taylor, 1977). The majority of farmers in the present study sprayed twice a week (33%) an indication of intense exposure of ticks to acaricides and of the 165 farmers that spray twice a week, 140 (85%) were from Eastern Province. Therefore, the phenomenon of intense acaricide application is more common in the eastern province and one of the leading factors that has led to reduced susceptibility of ticks to currently used acaricides.

## Conclusion

The management of acaricide resistance is a complex phenomenon that involves understanding the distribution of the resistance both by temporal and geographical distribution. The geographical distribution has to a great extent been elucidated in the present study: it is more in Eastern than Western provinces in Rwanda. The gaps in this area remain to study the phenomenon in Northern and Southern provinces. More importantly will be to apply tools that predict acaricide resistance for particular tick species and also cross resistance among acaricides. This can be achieved by applying molecular mechanisms for acaricide resistance. For instance, it is known that mutations in the sodium channel alleles are associated with pyrethroid resistance (Foil *et al.*, 2004) and metabolic detoxification of acaricides is known to be mediated by multigene- families of enzymes such as glutathione S-transferase (GST), esterases and mixed function oxidases (cytochrome P450) (Kyambay and Jewess, 2005). The basic mode of action of pyrethroids, therefore, is by disruption of sodium channel function. The choice of new acaricides therefore, should be guided by cross resistance studies and mechanisms of action for individual acaricides.

In this way, it will be possible to inform acaricide use policy / farmers on which acaricide classes to switch to when faced with reduced susceptibility or even resistance to acaricides in use. In fact, a tick control strategy for Rwanda should be developed if effective control of tick borne diseases is to be achieved.

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## CLINICAL PRESENTATION, TREATMENT AND MANAGEMENT OF SOME RABBIT CONDITIONS IN NAIROBI

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### Abstract

A retrospective study carried from records at the Small Animal Clinic, University of Nairobi between the years 1999 to 2010 to investigate the occurrence of rabbit conditions /diseases found a total of fifty clinical cases to have been presented within the period. Annual variations in the number of cases presented were noted with the highest incidence of 54% in the year 2010. The incidence of infectious conditions were ear canker 36% (18/50), gastrointestinal conditions 22% (11/50), pneumonia 12% (6/50) and skin conditions at 6% (3/50). Single cases of Ehrlichiosis, vaginal prolapse and Vitamin E / selenium deficiency were observed. Cases classified under routine health checks accounted for 18% (9/50) of the conditions. The distribution of the diseases by breed was New Zealand White 40% (20/50), California White 20% (10/50), crosses 24% (12/50), Dutch 10% (5/50), and Kenya White 6% (3/50). More female cases were noted relative to males at 78% (39/50) and 22% (11/50), respectively. A similar prevalence was also observed as per the age group where the adults were more than the kittens (baby rabbits). The mortality rate of the cases was 26% (13/50). Pneumonia had the highest case fatality rate at 83% (5/6) followed by gastrointestinal conditions 36% (4/11) and ear canker 22% (4/18). The findings of this study revealed that ear canker, intestinal coccidiosis and pneumonia were the most prevalent conditions affecting rabbits in Nairobi, Kenya.

**Keywords:** Rabbits diseases, ear canker, coccidiosis, pneumonia

## PRESENTATION CLINIQUE, TRAITEMENT ET GESTION DE CERTAINES MALADIES DU LAPIN A NAIROBI

### Résumé

Une étude rétrospective basée sur les dossiers de la Small Animal Clinic (clinique des petits animaux) de l'Université de Nairobi, effectuée entre les années 1999 et 2010 dans le but d'étudier la présence de maladies du lapin, a relevé un total de cinquante cas cliniques qui avaient été présentés durant cette période. Les variations annuelles du nombre de cas présentés ont été notées, l'incidence la plus élevée de 54% ayant été enregistrée en 2010. L'incidence des maladies infectieuses se présentait comme suit : le chancre de l'oreille 36% (18/50), les troubles gastro-intestinaux 22% (11/50), la pneumonie 12% (6/50) et les affections cutanées 6% (3/50). Des cas isolés d'ehrlichiose, de prolapsus vaginal et de carence en vitamine E / sélénium ont été relevés. Les cas classés au titre des examens médicaux de routine représentaient 18% (9/50) des maladies. La répartition des maladies par race se présentait comme suit : Blanc de Nouvelle-Zélande 40% (20/50) ; Blanc de Californie 20% (10/50) ; races croisées 24% (12/50) ; Néerlandais 10% (5/50) ; et Blanc du Kenya 6% (3/50). On a noté plus de cas chez les femelles par rapport aux mâles, respectivement 78% (39/50) et 22% (11/50). Une prévalence similaire a également été observée suivant le groupe d'âge où les adultes étaient plus nombreux que les lapereaux. Le taux de létalité était de 26% (13/50). La pneumonie avait le taux de létalité le plus élevé de 83% (5/6), suivie par les affections gastro-intestinales et le chancre de l'oreille respectivement avec des taux de létalité de 36% (4/11) et de 22% (4/18). Les résultats de cette étude ont révélé que le chancre de l'oreille, la coccidiose intestinale et la pneumonie étaient les maladies courantes affectant les lapins à Nairobi (Kenya).

**Mots-clés:** Maladies des lapins ; chancre de l'oreille ; coccidiose ; pneumonie

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## Introduction

Due to the decreasing land sizes as well as the increasing human population in the high potential areas of Kenya (Mutugi, 2004), many farmers have ventured into rabbit keeping as an alternative source of animal protein and for income generation (McNitt, 1980; Wanjaiya and Pope, 1985; Karikari and Asare, 2009).

Diseases such as coccidiosis, pneumonia and nutritional deficiencies have been reported as some of the main factors that constrain this enterprise worldwide (Langan *et al.*, 2000; Martino and Luzi, 2008). In Kenya, only postmortem cases have been documented (Ngatia *et al.*, 1988; Wesonga and Munda, 1992) with no documentation on clinical cases.

This paper seeks to address this by giving an overview of some of the clinical diseases of rabbits based on records at the Small Animal Clinic, University of Nairobi, Kenya.

## Materials and Methods

The data used for this study were from fifty clinical cases presented by rabbit keepers to the Small Animal Clinic, University of Nairobi over a period of eleven years from the year (1999 to 2010).

Using daily records, all rabbit cases were identified, and thereafter specific individual records scrutinized to ascertain the actual diagnosis, treatment, outcome and the diagnostic techniques employed. All data were entered and stored in Microsoft office Excel 2007 (Microsoft Corporation, 2007) and exported to SAS (Statistical Analytical System)® 2002 – 2003 (SAS Institute Inc., Cary, NC, USA) for analysis. Descriptive statistics based on sex, age, breeds, disease outcome and diagnosis were generated.

## Results

The results of the fifty rabbit clinical cases attended to in the Small Animal Clinic, University of Nairobi over the eleven year period are shown in Tables 1 and 2. There were annual variations in the number of cases presented to the clinic from year to year.

However, the number of cases had increased in the year 2007 and 2010, respectively as shown below in Table 1.

New Zealand White were the most common breed presented to the clinic at 40% (20/50). The other breeds were California White 20% (10/50), crosses 24% (12/50), Dutch 10% (5/50), and Kenya White 6% (3/50). More female cases were presented compared to the male at 78% (39/50) and 22% (11/50), respectively. A similar prevalence was also observed as per the age group where the adults were more than the kittens.

The mortality rate of the cases was 26% (13/50) where pneumonia had the highest case fatality rate at 83% (5/6) followed by gastrointestinal conditions 36% (4/11) and ear canker 22% (4/18).

Ear canker had the highest prevalence (36%) of the cases as a result of mite infestation (*Psoroptes cuniculi*). Localized mange on other body parts was also observed as only 6% of the cases. A minority 1% of skin conditions was due to dermatomycosis. Confirmatory diagnosis of skin conditions was done using skin scrapings that were digested using 5% potassium hydroxide (KOH) and observed under a light microscope. The treatment of mite infestation involved the use of ivermectin injection at 0.4 mg/kg bwt S.C. given 2 weeks apart with antibiotics depending on severity of the lesions and the presence of secondary infections.

The second most common conditions were those affecting the gastrointestinal tract at 22% (11/50) where majority were due to intestinal coccidiosis - 18% (9/50) and simple gastric bloat - 4% (2/50). Confirmatory diagnosis of intestinal coccidiosis was done using direct examination of fecal smears under a light-microscope where coccidia oocysts were observed. Lateral abdominal x-rays were indicated in cases of bloat. Use of potentiated sulphur drugs and metabolic stimulants (Catasol®) were used in the treatment of intestinal coccidiosis whereas bloat was treated using laxatives (liquid paraffin) and withholding of feed for 24 hours.

The third most common condition was pneumonia. Clinical signs (dyspnoea, harsh lung sounds) and lateral chest x-rays were the main diagnostic techniques employed. Such cases

**Table 1:** Distribution of rabbit clinical cases by year in the Small Animal Clinic, University of Nairobi between 1999 and 2010

Diagnosis or Condition	Year								Total (%)
	1999 -2003	2004	2005	2006	2007	2008	2009	2010	
Gastrointestinal conditions	-	1	-	-	2	-	-	8	11 (22%)
Skin conditions	-	-	-	-	2	-	-	1	3 (6%)
Ear canker	2	-	-	1	8	-	-	7	18 (36%)
Pneumonia	-	-	-	-	-	1	1	4	6 (12%)
Check-ups	-	2	2	-	-	1	-	4	9 (18%)
Other conditions	-	-	-	-	-	-	-	3	3 (6%)
<b>Total (%)</b>	<b>2 (4%)</b>	<b>3 (6%)</b>	<b>2 (4%)</b>	<b>1 (2%)</b>	<b>12 (24%)</b>	<b>2 (4%)</b>	<b>1 (2%)</b>	<b>27 (54%)</b>	<b>50</b>

**Table 2:** Prevalence of the rabbit conditions as observed in the Small Animal Clinic, University of Nairobi between 1999 and 2010 based on age, sex and survival rate

Diagnosis or Condition	Age-group		Sex		Outcome	
	Adults	Kittens	Males	Females	Survived	Died
Gastrointestinal conditions	11	-	1	10	7	4
Skin conditions	-	3	1	2	3	-
Ear canker	15	3	6	12	14	4
Pneumonia	6	-	1	5	1	5
Routine Check-ups	7	2	2	7	9	-
Other conditions	-	3	-	3	3	-
<b>Totals</b>	<b>39 (78%)</b>	<b>11 (22%)</b>	<b>11 (22%)</b>	<b>39 (68%)</b>	<b>37 (74%)</b>	<b>13 (26%)</b>

were treated using broad spectrum antibiotics and anti-inflammatory drugs. Pneumonia had the highest case fatality rate at 83%.

The category under check-up included cases that had been presented for routine health checks, sex identification, grooming, castration and or vaccination against rabies disease. Other minor clinical cases attended to under the category (other conditions) included a case of vaginal prolapse in a pregnant doe, Ehrlichiosis and hind limb paralysis due to vitamin E / selenium deficiency.

## Discussion

The results of this study indicate that clinical cases on rabbits had increased tremendously in the year 2010 compared to previous years. This trend could be attributed to the recent interest in rabbit keeping by many small-scale farmers in response to the

increasing human population, reduction in land size per capita holdings (Mutugi, 2004) and increased global demand for animal protein.

The three most predominant infectious conditions from this study were ear canker, gastrointestinal conditions (intestinal coccidiosis and gastric bloat) and pneumonia. This was partly in agreement with previous studies where respiratory and gastrointestinal conditions were the most common diseases (Ngatia *et al.*, 1988). This study showed that the occurrence of the above conditions was more common in the females and adults. This was not in agreement with other reports where kittens were at a higher risk of contracting infections due to their low immunity (Sicwaten, and Stahl, 1982) probably due to poor housing and managerial factors in the farm set-ups (Langan *et al.*, 2000).

A sizeable number of the cases observed were for routine health checks without any

complaint of illness, an indication of the rising interest and concern by the farmers for this new enterprise. However, a small percentage of the clients had interest on keeping the rabbits as pets which was concluded from the cases where the bucks were presented for castration, indicating a new and interesting trend.

A minor incidence of conditions categorized under other conditions included a single case of Ehrlichiosis, vaginal prolapse and selenium/vitamin E deficiency. There is need to therefore emphasize on ectoparasite control strategies and good nutrition.

The study showed that the most important diseases / conditions of rabbits included; mites causing ear canker and alopecia, intestinal coccidiosis and pneumonia. It is believed that these clinical cases will increase steadily in the future and in other tropical countries with similar environmental pressures. These rabbit conditions are preventable through sound management such as observing high standards of hygiene and proper housing designs. It is recommended that veterinarians in Kenya become keen on rabbit medicine and management due to the increased interest in rabbit keeping in the country.

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## STUDY OF SOME VIRULENCE FACTORS OF *E. COLI* FROM DIARRHEIC SHEEP AND GOATS.

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### Abstract

The aim of this study was to investigate some virulence factors of *E. coli* from diarrheic sheep and goats. *E. coli* was isolated at highest percentage from diarrheic sheep at age ranging from 1 to 6 months and highest percentage from diarrheic goats aged from 7 to 12 month.

*E. coli* isolated from diarrheic sheep gave alpha, beta and gamma hemolysis at percentage of 66.7 %, 18.2 % and 15.1%, respectively. Moreover, *E. coli* isolated from diarrheic goats gave alpha, beta and gamma hemolysis at percentage of 55%, 30% and 15 %, respectively. Results of Congo red test revealed that 90.9 % of *E. coli* isolated from diarrheic sheep and 90% isolated from goats gave positive results.

The results presented in table (7) revealed that serotype O26 was the most *E. coli* serotype (31.4% ) that gave a positive Congo red followed by *E. coli* serotype O78 (22.9%) and *E. coli* serotype O86 (17.1%).

Results of detection of verotoxin 2 gene of *E. coli* isolated from diarrheic sheep and goats revealed that the gene could not be detected in all examined *E. coli* isolates (7 isolates). On the other hand, results of detection STa gene in *E. coli* isolated from diarrheic sheep and goats revealed that 5 out of 7 *E. coli* isolates (71.4 %) were detected. *astA* gene of *E. coli* isolated from diarrhoeic sheep and goats was detected in all the examined isolates (7 isolates). It could be concluded that *E. coli* isolated from diarrhoeic sheep and goats was pathogenic and producer for enterotoxin and *astA*. Moreover, PCR is a simple and rapid method for detection of STa, *astA* and VT2 genes of *E. coli*

## ETUDE DE CERTAINS FACTEURS DE VIRULENCE DE LA BACTERIE *E. COLI* CHEZ LES MOUTONS ET CHEVRES DIARRHEIQUES

### Résumé

Le but de cette étude était d'examiner certains facteurs de virulence de la bactérie *E. coli* chez les moutons et chèvres diarrhéiques. Cet objectif a été atteint en déterminant les éléments suivants : Incidence de la bactérie *E. coli* dans les frottis rectaux prélevés chez des moutons et chèvres diarrhéiques ; Corrélation entre les isolats de l'*E. coli* en ce qui concerne l'âge ; Activité hémolytique des isolats de l'*E. coli*; Sérotypage des *E. coli* pathogènes ;

Détection de gènes d'entérotoxine, d'Asta et de vérotoxine des isolats de l'*E. coli* en utilisant la réaction en chaîne par polymérase ; AntibioGramme des isolats de l'*E. coli*.

Résultats : La bactérie *E. coli* a été isolée au plus grand pourcentage chez les moutons diarrhéiques d'un âge variant entre 1 et 6 mois ; tandis que pour les chèvres diarrhéiques le plus grand pourcentage a été noté chez celles âgées de 7 à 12 mois.

L'*E. coli* isolée chez les moutons diarrhéiques a donné une hémolyse alpha, bêta et gamma, respectivement aux pourcentages de 66,7%, 18,2% et 15,1%. En outre, l'*E. coli* isolée chez les chèvres diarrhéiques a donné une hémolyse alpha, bêta et gamma, respectivement aux pourcentages de 55%, 30% et 15%.

L'épreuve au rouge Congo a révélé que 90,9% des *E. coli* isolées chez les moutons diarrhéiques et 90% isolées chez les chèvres ont donné des résultats positifs.

Les résultats présentés dans le tableau (7) ont révélé que le sérotype O26 était le sérotype *E. coli* (31,4%) ayant donné un rouge Congo positif, suivi du sérotype O78 (22,9%) et du sérotype O86 (17,1%).

Conclusion : Les résultats de la détection du gène de la vérotoxine type 2 de l'*E. coli* isolée chez les moutons et les chèvres diarrhéiques ont révélé que le gène ne pouvait pas être détecté dans tous les isolats examinés de la bactérie (7 isolats). D'autre part, les résultats de la détection du gène STa dans l'*E.*

*E. coli* isolée chez les moutons et les chèvres diarrhéiques ont révélé que 5 des 7 isolats de l'*E. coli* (71,4%) ont été détectés. Le gène *astA* de l'*E. coli* isolée chez les moutons et les chèvres diarrhéiques a été détecté dans tous les isolats examinés (7 isolats). L'on pourrait conclure que la bactérie *E. coli* isolée chez les moutons et chèvres diarrhéiques était pathogène et produisait de l'entérotoxine et de l'*astA*. Par ailleurs, la PCR est une méthode simple et rapide de détection des gènes *STa*, *AstA* et *VT2* de la bactérie *E. coli*.

## Introduction

Verotoxin-producing *Escherichia coli* (VTEC) produce two families of verotoxins, VT1 and VT2 (Mainil, 1999). VTEC can cause severe diseases in humans such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). Those VTEC strains that are able to induce HC and HUS are called enterohaemorrhagic *E. coli* (EHEC) (Mainil, 1999). VTEC and EPEC have been associated with diarrhoea in goat kids (Orden et al., 2003).

Strains of *E. coli* produce protein toxins, many of which appear to play a role in disease process. These include alpha haemolysin, enterohaemolysin, verocytotoxins (Shiga-like toxins), cytotoxic necrotizing factor (CNF) and enterotoxins (Gyles, 1992).

EAST1 (Enterotoxin Aggregative heat-Stable Toxin I) is encoded by the *astA* gene, a 117 bp long DNA sequence (Savarino et al., 1991). EAST1 is thought to play a role in EAEC pathogenicity even if not all EAEC strains harbour the *astA* gene (Huang et al., 1994). Epidemiological studies have associated this gene with *E. coli* pathotypes other than EAEC like ETEC and EHEC and other bacteria genus including *Salmonella* (Savarino et al., 1996 and Paivade Sousa et al., 2001). The *astA* gene has also been detected in *E. coli* strains isolated from animal hosts including pigs, cattle, and sheep (Veilleux and Oubreuil et al., 2006).

Quinn et al., (2002) revealed that two types of enterotoxins, heat labile (LT) and heat stable (ST) have been identified each type of enterotoxin has two subgroups. Many strains of ETEC from pigs produce LT1. A second heat-labile toxin, LT2, has been demonstrated in some ETEC strains isolated from cattle. One of the heat-stable enterotoxin subgroups, *Sta*, has been identified in strains of ETEC isolated from porcine, bovine, ovine and human specimens.

PCR was available and sensitive method for determining the virulence factors of

*E. coli* strains and seemed to give good results in epidemiological investigation of diarrhoeogenic *E. coli* (Osek et al., 1999).

### Objective of the study

#### Materials:

A total of 163 rectal swabs were collected from sheep and goats. 112 rectal swabs from diarrhoeic sheep and 51 rectal swabs from diarrhoeic goats. The samples were collected, labeled and transported in ice box to the laboratory.

#### Media for bacteriological examination:-

Media used for primary isolation and identification of *E. coli* were prepared according to Cruickshank et al., (1975):

#### Biological reagents:

Antisera intended for the serological identification of *E. coli* were used.

#### Reagents and chemicals used for polymerase chain reaction (PCR):

#### Oligonucleotide primers:

The primers were selected to amplify *E. coli*:

- stable toxin *STa* gene (Ojeniyi et al., 1994). downstream primer (*STa2*) with a sequence of 5' ATAACATCCAGCACAGC 3' and 5' TCCGTGAAA CAACATGACGG 3'.
- EAST-1 downstream primer with a sequence of 5' TAGGATCCTCAGGTCGCGAGTGACGGC 3' and 5' TGC CATCAA CACAGTATATCC 3'.
- STX2 (*VT2*) downstream primer with a sequence of 5' GGA TGC ATC TCTGGT CAT TG 3' and 5' CTT CGG XXX CCT ATT CCG GC3. These primers were synthesized and supplied by Gibco BRL life Technologies Inc.

Ready –To-GOTM PCR Beads (Amersham pharmacia Biotech. Inc.):

These beads contain the following ingredients: Taq DNA polymerase, 10 x reaction Buffer (K<sub>2</sub>C 1500 mM and Tris-HCl 100 mM pH 9.0), 25 mM MgCl<sub>2</sub> and dNTP mix (10 mM of each dNTP).

#### Antibiotic discs:

The antibiotic discs and their concentration were presented in the following Table 1.

#### Collection of samples:

Rectal swabs were taken from diarrhoeic sheep and goats by means of sterile cotton swabs (Boyd *et al.*, 1974). The collected samples were transferred in ice bags to the laboratory where the samples were subjected to bacteriological examination as soon after.

#### Bacteriological examination:

Bacteria was identified according to Edwards and Ewing (1972); Finegold and Martin (1982); Koneman *et al.*, (1993) and Quinn *et al.*, (2002)

#### Serotyping of *E. coli*:

Slide agglutination test was carried-out for serotyping of *E. coli* according to Ewing (1986) as follows:-

#### Antimicrobial sensitivity test:

Antimicrobial resistance testing was performed by the disk diffusion method according to guidelines established by the National Committee for Clinical Laboratory Standards (Nccls, 2006).

Detection the gene coding enterotoxin and verotoxin of *E. coli* isolates using polymerase chain reaction

#### Bacterial template DNA:

DNA template was prepared from *E. coli* cells according to Nishikawa *et al.*, (1988).

#### Oligonucleotide primers set STa:

Primers were dissolved in nuclease-free water to obtain 50 – 100 p mol concentration (Sambrook *et al.*, 1989 and Baumforth *et al.*,

#### Oligonucleotide primers set SLT-II:

Primers were dissolved in nuclease-free water to obtain 50 – 100 p mole concentration. 5 µl of two primers were used in PCR mixture (Fratemico *et al.*, 2000).

#### Programming the thermal cycler:

i. Programming the thermal cycler for detection of STa gene: (Sambrook *et al.*, 1989; Baumforth *et al.*, 1999 and samer, 2001):

The thermal cycler (Biometra, personal cycler) was programmed and presented in Table (2).

ii. Programming the thermal cycler for detection of SLT-II gene: (Pina *et al.*, 2000):

The thermal cycler was programmed as presented in table (3).

## Results

Incidence of *E. coli* isolated from diarrheic sheep and goats at different ages

Results presented in Table 4 revealed the incidence of *E. coli* isolated from diarrheic sheep. *E. coli* was isolated at highest percentage from diarrheic sheep at age ranged from 1 to 6 month.

Results presented in Table 5 revealed the incidence of *E. coli* isolated from diarrheic goats. *E. coli* was isolated at highest percentage from diarrheic goats at age ranged from 7 to 12 month.

Results of *E. coli* isolated from sheep and goats were presented in Table 6. *E. coli* was isolated at the highest percentage from sheep and goats at 7-12 month old.

Results of haemolysis produced by *E. coli* isolated from Diarrheic sheep and goats

Results presented in Table 7 show that *E. coli* isolated from diarrheic sheep gave alpha, beta and gamma hemolysis at percentage of 66.7 %, 18.2 % and 15.1%, respectively. More over, *E. coli* isolated from diarrheic goats gave alpha, beta and gamma hemolysis at percentage of 55%, 30% and 15 %, respectively.

**Table 1:** The antibiotic discs and their concentration

Antibiotic	Concentration
Colistin (CT )	10 ug
Neomycin (N)	30 ug
Marbofloxacin (MAR)	30 ug
Tulathromycin (TUL)	30 ug
Levofloxacin ( LEV )	5 IU
Norfloxacin (NOR)	10 IU
Ofloxacin (OFX)	5 IU
Trimethoprim/Sulfamethoxazole (SXT)	25 ug
Gentamicin (CN )	10 ug
Tobramycin (TOB )	10 IU

**Table 2:** Setting up the thermal cycler for STa gene:

No. of cycles	°C	Time / min	Target
1 cycle	94	5	DNA template denaturation
30 cycles of	94	1	Denaturation
	57	1	Primer annealing
1 cycle	72	2	Extension
1 cycle	72	10	Final extension
	4	∞	preservation

**Table 3:** Setting up the thermal cycler for SLT-II gene:

No. of cycles	°C	Time	Target
1 cycle	94	2 min	DNA template denaturation
35 cycles of	94	20 sec.	Denaturation
	57	1 min.	Primer annealing
	72	1 min	Extension
1 cycle	72	10 min	Final extension
1 cycle	4	∞	preservation

**Table 4:** Results of E. coli isolated from diarrheic sheep at different ages.

Age of sheep	No. of examined samples	No. of E. coli isolates	%
From (1) to (6 ) month	72	23	31.94
From (7) to (12 ) month	29	7	24.14
Over one year	11	3	27.27
<b>Total</b>	<b>112</b>	<b>33</b>	<b>29.5</b>

**Table 5:** Results of E. coli isolated from diarrheic goats at different ages.

Age of animal	No. of examined samples	No. of E. coli isolates	%
From (1) to (6 ) month	23	5	21.7
From (7) to (12 ) month	14	11	78.6
Over (1) year	14	4	28.6
<b>Total</b>	<b>51</b>	<b>20</b>	<b>39.2</b>

**Table 6:** Results of E. coli isolated from diarrheic sheep and goats at different ages.

Age of animal	No. of examined samples	No. of E. coli isolates	%
From (1) to (6 ) month	95	28	29.5
From (7) to (12 ) month	43	18	41.9
Over (1) year	25	7	28
<b>Total</b>	<b>163</b>	<b>53</b>	<b>32.5</b>

**Table 7:** Results of haemolysis produced by E. coli isolated from Diarrheic sheep and goats.

Animal species	No. of E. coli isolates	Alpha hemolysis		Beta hemolysis		Gamma hemolysis	
		No.	%	No.	%	No.	%
Sheep	33	22	66.7	6	18.2	5	15.1
Goats	20	11	55	6	30	3	15
Total	53	33	62.3	12	22.6	8	15

**Table 8:** Results of Congo red test of E. coli isolated from diarrheic sheep and goats.

Animal species	No. of E. coli isolates	Positive Congo red	
		No. of isolates	%
Sheep	33	30	90.9
Goats	20	18	90
<b>Total</b>	<b>53</b>	<b>48</b>	<b>90.6</b>

Results of Congo red test of E. coli isolated from Diarrheic sheep and goats.

Results of Congo red test revealed that 90.9% of E .coli isolated from diarrheic sheep and 90% E. coli isolated from goats gave positive results (Table, 8).

Relationship between E. coli serotypes and its Congo red test.

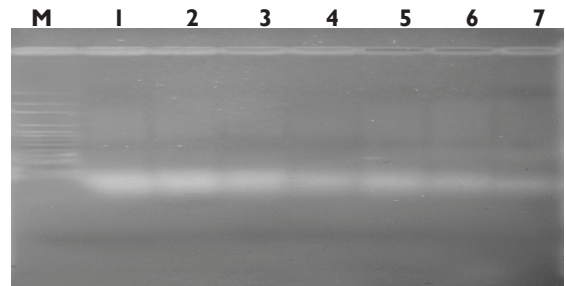
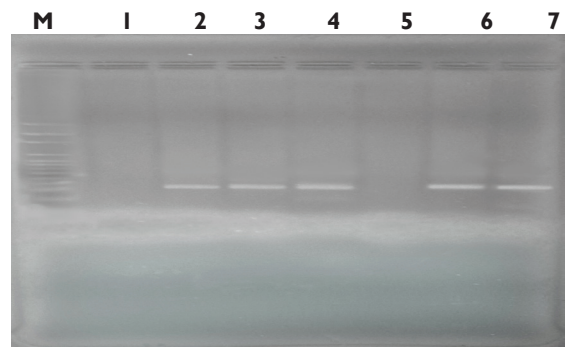
The results presented in Table 10 revealed that E. coli serotype O26 was the most E. coli serotype (31.4% ) that gave a positive Congo red followed by E. coli serotype O78 (22.9%) and E. coli serotype O86 (17.1%).

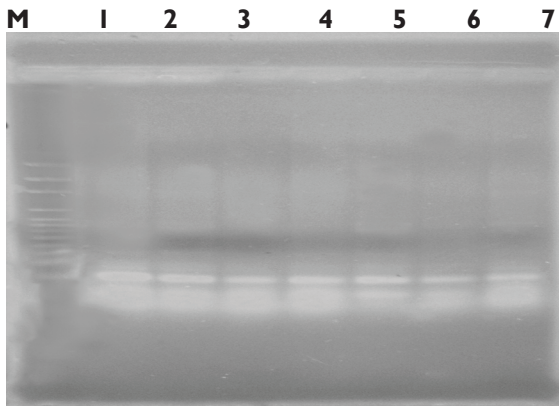
Results antibiotic sensitivity test of E. coli isolated from diarrheic sheep and goats.

Results of antibiotic sensitivity test of E.coli isolated from diarrheic sheep and goats indicated that levofloxacin , norfloxacin and ofloxacin were the most effective antibiotics (Table, 1)

Results of detection of verotoxin 2 gene of E. coli isolated from diarrheic sheep and goats.

Results of detection of verotoxin 2 gene of E. coli isolated from diarrheic sheep and goats revealed that the gene could not

**Fig. 1:** Results of amplification verotoxin 2 gene by PCR. Lane M: DNA molecular weight marker. Lanes 1, 2, 3, 4, 5, 6 and 7 were negative E. coli isolates for VT2 gene.**Fig. 2:** Results of amplification STa gene by PCR. Lane M: DNA molecular weight marker. Lanes 2, 3, 4, 6 and 7 were positive E. coli isolates for STa gene while lanes 2, 1 and 5 were negative.



**Fig. 3:** Results of amplification astA gene by PCR. Lane M: DNA molecular weight marker. Lanes 1, 2, 3, 4, 5, 6 and 7 were positive *E. coli* isolates for astA.

be detected in all examined *E. coli* isolates (7 isolates) (Figure. 1).

*Results of detection of STa gene of E. coli isolated from diarrheic sheep and goats.*

Results of detection STa gene of *E. coli* isolated from diarrheic sheep and goats revealed that 5 out of 7 (71.4%) were positive (Figure.2).

*Results of detection of EASTI gene of E. coli isolated from diarrheic sheep and goats.*

Results of detection EAST-I gene of *E. coli* isolated from diarrheic sheep and goats revealed that all the examined isolates (7 isolates) were positive (Figure. 3).

**Discussion**

Results presented in Table (4, 5 and 6) showed the incidence of *E. coli* isolated from diarrheic sheep and goat. *E.coli* was isolated at highest percentage from diarrheic sheep at age ranged from 1 to 6 month. The age factor played a role in the incidence of *E. coli* where the recovery among cattle calves and buffalo calves was relatively lower to 11.9% and 21.4%, respectively at age of 1 – 3 months, 8.8% and 15.7% respectively at age of 3 – 6 months. Similar observation was recorded by ( Asma et al., 1996 and Abd El Kader et al., 2001).

Results presented in Table 7 show that *E. coli* isolated from diarrheic sheep gave alpha,

beta and gamma hemolysis at percentage of 66.7 %, 18.2 % and 15.1%, respectively. Moreover, *E. coli* isolated from diarrheic goats gave alpha, beta and gamma hemolysis at percentage of 55%, 30% and 15 %, respectively. These results are coincident with those described by Samer (2001) reported that only 4.91% (9/140) of the *E. coli* isolates from diarrhoeic animals were  $\alpha$ -Hly producing *E. coli*. and he added that, there is no association between  $\alpha$ -Hly and VT production. The haemolytic *E. coli* isolates used as a phenotypic marker or virulence factor of *E. coli* isolates associated with diarrhoea. The 2 *E. coli* O157 isolates and the 6 haemolytic *E. coli* isolates (one of 7 haemolytic *E. coli* isolates was *E. coli* O157) and randomly 6 non-haemolytic *E. coli* isolates considered three groups (Gaber, 2002).

Results of Congo red test showed that 90.9% of *E .coli* isolated from diarrheic sheep and 90% *E. coli* isolated from goats gave positive results (Table, 8). Alskan (1995) observed that Congo red positive *E. coli* cultures bind enough Congo red dye to obtain a red colony after 24 hours of incubation and that Congo red test may be used as a screening test for detection of virulent *E. coli*. Congo red dye agar test can be used for primary screening of non invasive *E. coli* from potentially invasive *E. coli* (Moussa et al., 2006).

The results presented in Table (10) revealed that *E. coli* serotype O26 was the most *E. coli* serotype (31.4% ) that gave a positive Congo red followed by *E. coli* serotype O78 (22.9%) and *E. coli* serotype O86 (17.1%).

The results of antibiotic sensitivity of *E.coli* to chemotherapy revealed that *E.coli* was sensitive to levofloxacin, norfloxacin, ofloxacin and trimethoprim/sulfamethoxazole. On the other hand *E.coli* was resistant to colistin and neomycin (Table, 11). These results agree with those obtained by Aisha and Youseif (1999) who found that all *E. coli* isolates were sensitive to gentamycin, enrofloxacin and in sulphate. Glisson et al., (2004) posted that chickens that received enrofloxacin had significantly less mortality, lower average gross pathology scores, and better feed conversion ratios than did chickens that received either oxytetracycline or no medication. They also reported that chickens which received

**Table 9:** Relationship between E. coli serotypes and its hemolytic activities.

Serotypes of E. coli	Alpha hemolysis E. coli		Betahemolysis E. coli		Gamma hemolysis E. coli	
	No.	%	No.	%	No.	%
O 114	1	2.9	0.0	0.0	0.0	0.0
O 55	2	5.7	0.0	0.0	0.0	0.0
O 78	4	11.4	2	5.7	3	8.6
O 25	0.0	0.0	1	2.9	0.0	0.0
O 158	1	2.9	0.0	0.0	0.0	0.0
O 86	3	8.6	3	8.6	0.0	0.0
O 26	8	22.6	1	2.9	2	5.7
O 128	4	11.4	0.0	0.0	0.0	0.0
<b>Total</b>	<b>23</b>	<b>65.7</b>	<b>7</b>	<b>20</b>	<b>5</b>	<b>14.3</b>

**Table 10:** Relationship between E. coli serotypes and Congo red.

Serotypes of E. coli	Results of Congo red test			
	Positive		negative	
	No	%	No	%
O 114	1	2.9	0.0	0.0
O 55	2	5.7	0.0	0.0
O 78	8	22.9	1	2.9
O 25	1	2.9	0.0	0.0
O 158	1	2.9	0.0	0.0
O 86	6	17.1	0.0	0.0
O 26	11	31.4	0.0	0.0
O 128	4	11.4	0.0	0.0
<b>Total</b>	<b>34</b>	<b>97.1</b>	<b>1</b>	<b>2.9</b>

The percentage was calculated according to total No. of serotyped E. coli (35 isolates).

**Table 11:** results of chemotherapy sensitivity of E. coli isolated from diarrheic sheep and goats.

No of .coli isolates	Antibiotic	Sensitive		Intermediate		Resistant
		No.	%	No.	%	No.
<b>35</b>	Colistin (CT 10)	1	2.86	3	8.57	31
	Neomycin (N 30)	5	14.3	22	62.9	8
	Marbofloxacin (MAR)	10	28.6	4	11.4	21
	Tulathromycin (TUL30)	10	28.6	12	34.3	13
	Levofloxacin (LEV 5)	30	85.7	2	5.7	3
	Norfloxacin (NOR 10)	29	82.9	2	5.7	4
	Ofloxacin (OFX5)	30	85.7	2	5.7	3
	Trimethoprim/Sulfamethoxazole (SXT 25)	26	74.3	1	2.8	8
	Gentamicin (CN 10)	17	48.6	13	37.1	5
	Tobramycin (TOB 10)	10	28.6	8	22.9	17

**Table 12:** Correlation between *E. coli* serotypes ,VT2 ,Sta and astA genes.

E. coli serotype	No. of examined serotype	VT2	STa	astA
O25	1	Negative	Negative	Positive
O55	1	Negative	Positive	Positive
O26	1	Negative	Positive	Positive
O158	1	Negative	Positive	Positive
O128	1	Negative	Negative	Positive
O114	1	Negative	Positive	Positive
O78	1	Negative	Positive	Positive

enrofloxacin had significantly less mortality, lower pathology scores than those that received sulfadimethoxine and numerically feed conversion than the sulfadimethoxine group. Moreover, *E. coli* isolates from diseased chickens diagnosed with colibacillosis in Henan province, China were resistance to trimethoprim-sulfamethoxazole (100%), oxytetracycline (100%), ampicillin (83%), enrofloxacin (83%) and ciprofloxacin (81%).

Results of detection of verotoxin 2 gene of *E. coli* isolated from diarrheic sheep and goats revealed that the gene could not be detected in 7 examined *E. coli* isolates. These results match with those described by Heuvelink *et al.*, (1998) who found that verocytotoxin producing *E. coli* (VTEC) produces either or both of two phage-encoded toxins VT1 and VT2. Verotoxins are thought to cause vascular endothelial damage which observed by haemorrhagic colitis and haemolytic uraemic syndrome. An additional virulence factor contributing to the pathogenicity of VTEC is the formation of attaching and effacing lesions in the intestine of the host. Verocytotoxin (VT)-producing *E. coli* (VTEC) of different serotypes have become a major concern in human and animals diseases in different countries in the few years (Mohamed *et al.*, 1985; Ryan *et al.*, 1986; Karamali, 1989; Kleanthous *et al.*, 1990; Gyles, 1992; Wieler *et al.*, 1992). Begum *et al.*, 1993 assessed PCR technique for the identification of Shiga-like toxin (SLT) producing *E. coli*.

The role of enterohaemolysin in the pathogenesis of VTEC infection is not known (Chart *et al.*, 1998). There are no data on human infections and the toxin may be more important in assisting infection of animals, (Law,

2000).

Results of detection STa gene of *E. coli* isolated from diarrheic sheep and goats revealed that 5 out of 7 (71.4%) were positive. Pathogenic *E. coli* strains may produce a variety of toxins with different activities: heat-labile (LT) and heat-stable (ST) enterotoxins, shiga toxins and cytotoxic necrotizing factors 1 and 2 (CNF1 and CNF2). Shiga toxins are produced by EHEC strains (Riley *et al.*, 1983 and Quinn *et al.*, 2002 ). Enterotoxigenic strains usually produce two enterotoxins, LT and ST, which have distinct roles in the pathogenesis. *E. coli* isolates producing CNF2 are common in cattle (Pohl *et al.*, 1993). Many of these strains also produce F17b fimbriae and aerobactin and are resistant to the killing action of serum.

Results of detection EAST-1 gene of *E. coli* isolated from diarrheic sheep and goats revealed that all the examined isolates 8 were positive. The present results agree with those mentioned by Law (2000) who cited that the high prevalence of the astA genotype in O157 isolates is striking, indeed the incidence is considerably higher than that found in EAaggEC. He added that in *E. coli* O157 infection, it is possible that EAST1 contributes to the initial phase of watery diarrhoea seen in patients with HC.

The virulence of *E. coli* is multifactorial and contain properties associated primarily with virulent strains. One of these is the ability to produce a haemolysin (Cavaliere *et al.*, 1984). The  $\alpha$ -haemolysin is believed to be a virulence factor (Bohach and Snyder, 1985). The production of enterohaemolysin by some serogroups of *E. coli* was closely associated with (VT) production (Beutin and Montengro, 1990).

The study aimed to detect the gene coding for the production of heat stable enterotoxin (STa) and Shiga-like toxin type 2 (SLT-II) by three groups of *E. coli* isolates using PCR. As well as, to detect the gene expression by infant mouse assay and vero cell assay.

SLT production by non-O157 SLTEC is similar to that for serotype O157, namely that the SLTS are phage – encoded and strains may produce either one or both SLT. I and SLT. II (Marguerite, 1996).

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## SEROPREVALENCE OF INFECTIOUS BURSAL DISEASE IN BACKYARD CHICKENS OF NORTH WEST ETHIOPIA

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### Abstract

A cross sectional study was conducted in North Gondar and West Gojjam Administrative Zones from November 2009 to June 2010 to determine the seroprevalence of infectious bursal disease by using I-ELISA (Indirect enzyme linked immunosorbent assay) test. A total of 400 chickens raised in the back yard production system, 200 from each study area, were randomly selected and examined for the presence of anti-IBD (anti- infectious bursal disease) antibody. Anti-IBD antibody was detected from 294 chickens and this gives an overall seroprevalence of 73.5% (294/400) for the entire study area, where the higher 75% (150/200) and the lower 72% (144/200) was recorded from samples collected in West Gojjam and North Gondar, respectively. Even though, place of origin and sex was considered as potential risk factors, the study result shows that variation in place of origin and sex of chickens did not have significant influence on the occurrence of IBD. Generally, the higher prevalence (73.5%) reported in this study indicates that the infection is widely distributed and one of the potential threat for poultry production in the study areas. Therefore, further studies on the identification of serotype(s) should be initiated to enhance the control of the disease in the study area.

**Key words:** back yard, indirect ELISA, infectious bursal disease, North Gondar, Seroprevalence, West Gojjam

## SEROPREVALENCE DE LA BURSITE INFECTIEUSE CHEZ LES POULETS DE BASSE-COUR DANS LE NORD-OUEST DE L'ETHIOPIE

### Résumé

Une étude transversale a été menée dans les zones administratives de Gondar Nord et Godjam Ouest, de novembre 2009 à juin 2010, dans le but d'y déterminer la séroprévalence de la bursite infectieuse en utilisant l'épreuve I-ELISA (épreuve immuno-enzymatique indirecte). Au total, 400 poulets de basse-cour, dont 200 de chaque zone d'étude, ont été choisis de façon aléatoire et examinés en vue de rechercher la présence d'anticorps anti-BI (anti bursite infectieuse). L'anticorps anti-BI a été détecté chez 294 poulets, ce qui représente une séroprévalence globale de 73,5% (294/400) pour la zone d'étude, où des taux supérieurs de 75% (150/200) et inférieurs de 72% (144/200) ont été enregistrés respectivement pour les échantillons prélevés dans Godjam Ouest et dans Gondar Nord. Même si le lieu d'origine et le sexe étaient considérés comme des facteurs de risque potentiels, le résultat de l'étude montre que la variation du lieu d'origine et du sexe des poulets n'a pas d'influence significative sur la présence de la bursite infectieuse. En général, la forte prévalence (73,5%) rapportée dans cette étude est une indication que l'infection est largement répandue et constitue l'une des menaces potentielles à la production de volailles dans les zones d'étude. Par conséquent, il faudrait lancer des études approfondies sur l'identification du (des) sérotype(s) afin d'améliorer le contrôle de la maladie dans la zone d'étude.

**Mots-clés :** arrière-cour ; ELISA indirecte ; bursite infectieuse ; Gondar Nord ; séroprévalence ; Gojjam Ouest

## Introduction

Poultry breeding in Ethiopia has a long traditional practice; women are more involved in keeping back yard chickens for eggs and selling adult chickens. This extensive production practice has significant role in the livelihood of the farmers (Dawit *et al.*, 2008). Meanwhile, there has been a gradual decline in Ethiopian poultry production, according to Burley (1957) and the Central Statistical Agency (2008), the Ethiopian poultry population was estimated at 85 and 38.13 million in 1954 and in 2008, respectively.

Many biological and socio-economical factors are incriminated for the decrease in the poultry population in Ethiopia, of which disease and poor animal health service are the two most important factors. Among the different diseases causing damage in the poultry production in the country is infectious bursal disease (IBD) (FAO, 2008).

Infectious bursal disease is highly contagious immunosuppressive disease caused by a virus of the genus *Avibirnavirus* of the family *Birnaviridae* (Van Den Berg, 2000; Meihong and Vikram, 2004; Herdt *et al.*, 2005; OIE, 2008). It has been described throughout the world, and its socio-economic significance is recognized worldwide (Muller *et al.*, 2003), occurring in more than 95% of member countries of OIE (Van den Berg, 2000).

The report of introduction and existence of IBD in Ethiopia has come recently with the report of IBD outbreak in Debrezeit large scale poultry farms in the year 2002, where an overall of 49-89% mortality rate of chickens and 90.30% seroprevalence of IBD antibody were reported in different farms (Zelege *et al.*, 2003). Hailu *et al.*, (2009) also documented incidence rate of 38.4 and 17.4% in two localities namely Bahir Dar and Farta, respectively in an outbreak of IBD. However, the status of the disease in free ranging backyard chickens in the country is not yet well documented. Therefore, the objective of this study was to determine the seroprevalence of the disease in local free ranging backyard chickens.

## Materials and Methods

### Study area description

The study was conducted in North Gondar and West Gojjam Administrative Zones of Amhara National Regional State in the North Western part of Ethiopia from November 2009 to June 2010. West Gojjam Administrative Zone is situated in western part of Amhara National Regional State at an altitude range of 1500-2300 meters above sea level with mean annual rainfall of 1200-1600 mm and mean temperature of 10-20°C. North Gondar Administrative Zone is located in North west part of the same region and has an altitude that ranges from 4620 meters in the Semen Mountain in the North to 550 meters in the west. The rainfall varies from 880 mm to 1772 mm, while the minimum and maximum temperatures are in the order of 10°C in the highland and 44.5°C in the West. The farming system in these study areas is characterized by a mixed crop-livestock production system (Bureau of Agriculture, 2006).

### Study design and sampling procedures

A cross sectional study was conducted to determine the seroprevalence of infectious bursal disease in unvaccinated backyard chickens in 40 villages of the 6 districts found in West Gojjam and North Gondar Administrative Zones. These two zones were selected purposively because they represented the different agro-ecological zones of the region along with the dominant backyard production system and great potential for commercial poultry production. Multistage sampling technique was implemented to select districts and villages (Kebele: small administrative units in Ethiopia) from each zone.

Three districts and twenty villages were selected from each zone and 10 chickens from each village. A total of 400 unvaccinated backyard chickens with age greater than 3 weeks were randomly selected and blood sample was taken from each bird.

Sterile 3 ml/cc disposable syringe with needle size 22 (gauge) x 1 ¼" were used to collect about 2-3 ml of blood sample from wing (brachial) vein of chickens. A method described by Alcorn (2001) for intravenous technique was followed in this procedure. The sera poured

off from the syringes in to sterile eppendrof tubes were subjected to centrifugation at 1000 rpm for clarification. Each sample was labeled accordingly and the code was directly translated to eppendrof tubes holding the clarified sera. Then the clarified sera were stored at -20°C until tested at the national veterinary institute, Debre Zeit Ethiopia.

#### Test procedure and interpretation

In this study, indirect Enzyme-Linked Immunosorbant Assay (ELISA) commercially available Proflock plus infectious bursal disease virus antibody test kit was employed and the kit manufacturer procedure was followed.

Valid IBD ELISA results are obtained when the average optical density (OD) value of the normal control serum is less than 0.250 and the corrected positive control value range is between 0.250 and 0.900. If either of these values are out of range, the IBD test result should be considered invalid and the samples should be retested. OD value range of normal control serum was between 0.07-0.2 and for positive control serum 0.45-0.82.

The IBD ELISA titer values obtained represents a comparison of the IBD antibody level within each filed chicken serum tested and the IBD ELISA kit positive and non reactive sera. Therefore, it was important first to determine that the IBD ELISA positive and normal positive control sera values obtained are valid as detailed above in the “Assay Control Values” section. For interpretation of the test results, a Sp (sample to positive ratio) of each test serum was required. Then the sample to positive ratio calculated by the following formula directed by the manufacturer:

$$SP = \frac{\text{sample absorbance} - \text{average normal control absorbance}}{\text{Corrected positive control absorbance}}$$

An IBD ELISA titer calculated by the following suggested equation by the manufacturer

$$\text{Log}_{10} \text{ Titer} = (1.172 \times \text{Log}_{10} \text{ Sp}) + 3.614$$

then,

$$\text{Titer} = \text{Antilog of Log}_{10} \text{ Titer}$$

If Sp (sample to positive control) value was  $\geq 0.5$  the IBD antibody status was considered to be positive but  $< 0.5$  was taken as negative.

#### Data management and analysis

The data collected were entered and managed in Microsoft excel. Stata 11 software statistical program was employed for the data analysis. The prevalence of IBD was determined by dividing the number of positive serum samples by the total number of chicken serum samples tested for IBD, and was expressed as percentage. Chi-square test was used to assess if there was a statistically significant difference in IBD infection between sex groups and among different locations. For this analysis P-value less than 0.05 was considered significant whereas P value greater than 0.05 considered non significant.

## Results

Of the total 400 serum samples collected 294 samples were found positive for IBD yielding an overall prevalence of 73.5% for the study area. The prevalence in West Gojjam and North Gondar was 75% and 72%, respectively (Table 1). Though there was difference in seroprevalence of IBD between these two study areas, the difference was not statistically significant ( $P > 0.05$ ).

The seroprevalence of IBD in districts of West Gojjam ranges from 72-78%. The lowest and the highest seroprevalence were recorded from Mecha and Bahir Dar Zuria, respectively. The proportion of seropositive chickens, however, doesn't vary significantly among the three districts ( $P > 0.05$ ) (Table 2).

The seroprevalence of IBD from the three districts of North Gondar ranged from 70-73%. The lowest seroprevalence were detected from Dembya and the highest from Gondar town. The difference in the frequency of detection of IBD antibody, however, did not vary significantly among the three districts of North Gondar too ( $P > 0.05$ ) (Table 3).

**Table 1:** Seroprevalence of IBD in the two study areas

Study area	No. Samples examined	No. Positive	Prevalence (%)
West Gojjam	200	150	75
North Gondar	200	144	72
<b>Total</b>	<b>400</b>	<b>294</b>	<b>73.5</b>

**Table 2:** Seroprevalence of IBD in West Gojjam

Districts	No. Samples examined	No. Positive	Prevalence (%)
Bahir Dar town	100	75	75
Bahir Dar Zuria	50	39	78
Mecha	50	36	72
<b>Total</b>	<b>200</b>	<b>150</b>	<b>75</b>

**Table 3:** Seroprevalence of IBD in North Gondar

Districts	No. Samples examined	No. Positive	Prevalence (%)
Gondar town	100	73	73
Dembya	50	35	70
Gondar Zuria	50	36	72
<b>Total</b>	<b>200</b>	<b>144</b>	<b>72</b>

**Table 4:** Seroprevalence of IBD between sex groups

Sex	No. Samples examined	No. Positive	Prevalence (%)
Male	236	171	72.5%
Female	164	123	75.5%
<b>Total</b>	<b>400</b>	<b>294</b>	<b>73.5</b>

In this study, assessment was made to see the effect of sex on the IBD seroprevalence. Relatively higher seroprevalence was recorded among female chickens (75.5%) than that of male ones (72.5%), but the difference between sex groups was not statically significant ( $P>0.05$ ) (Table 4).

**Discussion**

The result of the current study demonstrates higher prevalence (73.5%) of IBD in unvaccinated local breed back yard chickens, indicating IBD is wide spread in the study areas. The presence of anti-IBDV (anti-infectious bursal disease virus) antibodies in the sera of unvaccinated chickens was evidence for the circulation of the virus and subsequent exposure of chickens in the field.

Even though it is documented that IBD is a primarily potential threat for the

commercial farms, different authors report evidence of the importance of the disease in the loose management system where chickens are usually kept without confinement. The finding of higher seroprevalence in this production systems is therefore, not unexpected and our finding was in agreement with the finding of Abrar (2007), who reported a prevalence of 76.3% in selected areas of East Showa Zone and Nigussie (2007), who reported a prevalence of 65.9% in non-vaccinated backyard chickens in Addis Ababa and Adami Tulu areas using ELISA test. This finding was also comparable to the finding of Ibrahim and Tanya (2001), who reported 60.6% prevalence in Nigeria from village chickens and Karunakaran *et al.*, (1993), who reported a prevalence of 73.8% in India by using ELISA test.

The finding of this study (73.5%) was higher than the finding of Reta (2008), who reported a prevalence of 39.2% in unvaccinated

backyard chickens in East Shoa Zone using AGID (Agar gel immuno-diffusion) test. Studies from abroad by different authors also indicate relatively lower prevalence like 49.3% by Ndanyi *et al.*, (2004) in Kenya, 34% Anjum *et al.*, (1993) in Pakistan and 45% by Tsai and Lu (1993) in Taiwan. The difference in the present results might be attributed to the difference in the test employed, serological survey results can vary depending on sensitivity and specificity of the diagnostic tool applied (DeWit *et al.*, 2007) and ELISA test is known to be highly sensitive than that of AGID (OIE, 2008).

The prevalence between the two study areas was found to be 75% and 72%, where the higher prevalence was observed in West Gojjam and the lower prevalence in North Gondar. District wise, the prevalence ranges from 70 to 78%, where the lower and higher prevalence was observed from samples collected in Dembya and Bahir Dar Zuria districts, respectively. Though there is variation in prevalence between the study areas and among districts, the difference was not statistically significant ( $P > 0.05$ ). This finding was inline with the finding by Nigussie (2007), who reported that the absence of variation in prevalence of IBD in different areas. This is also in agreement with the nature of the disease, as there is no specific environmental situation that can prevent or modify the occurrence of the disease. The disease occurs worldwide in all major poultry production areas and it can be serologically evident in all age groups (Van Den Berg, 2000) and IBD is very resistant to different environmental condition and it is capable of surviving in the environment for long period (Dawit *et al.*, 2007). The relatively higher Prevalence in Bahir Dar Zuria district might be associated with the introduction and establishment of the disease at Andasa poultry breeding and multiplication center (Woldemariam and Wossene, 2007), which is located in the district.

In this study relatively higher prevalence was recorded in female chickens (75.5%) than male ones (72.5%). However, the difference was not statistically significant ( $P > 0.05$ ). This finding was similar with that of Reta (2008), who reported the absence of influence of sex on the prevalence of the disease.

Although IBD is considered a problem of commercial poultry production system the current study revealed the widespread nature of the virus in the backyard production system. In commercial production system, where a lot of chickens exist, high morbidity with a spiking death curve may attract the attention of the professionals. Nevertheless, death of two or three chickens per household in the backyard production will leave behind the importance slight. Moreover, the immune suppressive form of the disease keeps chickens susceptible to different diseases such that many chickens will die regularly as the virus continues to circulate in the environment. Hence, the disease will seriously affect the livelihoods of the farmers in particular and the national economy in general, as the majority of the Ethiopian poultry population is found in the extensive scavenging production system (Central Agricultural Census Commission, 2008).

Though, it is described that IBD introduced recently in to the country by Zeleke *et al.*, (2005) the higher prevalence of the disease in the backyard production system will be indicative of the speed of spread of the disease. Governmental farms that distribute chickens to the farmers through the extension service may contribute for the introduction and spread of the disease in the backyard production system. Therefore, great emphasis has to be accorded particularly to the farms that distribute chickens and effective biosecurity, vaccination, follow up studies and frequent testing has to be made to diagnose the infection as soon as possible before distribution it to the farmers.

It is concluded that although IBD was considered less important in the backyard production system, the present study bare higher seroprevalence of the infection, 73.5% in the study areas signifying the infection was wide spread in backyard chickens and one of the potential threats to poultry production in the study areas. Variation in place of origin and sex of chickens does not have significant association with the occurrence of the infection. Therefore, this study necessitates further studies on the identification of serotype(s) to design and execute appropriate control measures.

## Impact

This study demonstrated the wide circulation of IBD virus in backyard chickens of North Gondar and West Gojjam administrative zones, contrary to belief that IBD is dominantly a problem of commercial poultry production systems. This information is very important for different stake holders to know about the existing status of IBD in the study area and initiate them to take the appropriate measures in order to control and eradicate the disease to reduce its economic burden.

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# ENQUÊTE SUR LA CHIMIORÉSISTANCE DES TRYPANOSOMES CHEZ LES BOVINS N'DAMA DANS LA ZONE COTONNIÈRE DE LA HAUTE GUINÉE (CAS DE LA PRÉFECTURE DE MANDIANA EN GUINÉE, CONAKRY)

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## Resume

Une enquête sur la chimiorésistance des trypanosomes chez les bovins N'dama a été menée en Haute Guinée. Le but de cette étude, a été d'estimer l'importance et la distribution de la chimiorésistance à Mandiana. A cet effet, un traitement en bloc utilisant du chlorure d'isométymidium (ISMM) dosé à 1mg/kg de poids vifs a été réalisé dans les villages de Saladou, Kanifra, Dialakoro. Les animaux du groupe traité au chlorure d'isométymidium ayant été positifs au BCT ont été blanchis à l'acéturate de diminazène à la dose de 3,5mg/kg de poids vifs pour les infections à *Trypanosoma congolense*, *T. vivax* et 7mg/kg (*T. brucei*). Une proportion faible de rechute à l'ISMM variant de 2 à 6% a été observée pendant le suivi longitudinal des animaux qui a duré 56 jours à raison d'un BCT chaque 2ème semaine. Aucun cas de rechute à l'acéturate de diminazène n'a été mis en évidence. Un second traitement en bloc été effectué pour voir l'importance des rechutes autour des villages de Saladou et Dialakoro. C'est ainsi que 10 villages à proximité de Saladou et 5 à Dialakoro ont été sélectionnés. Trois cas de rechutes dans 3 différents villages entourant Saladou ont été observés à l'intérieur des 28 jours de suivi des animaux. Pour préciser la nature des infections observées après les traitements en bloc à Saladou, Kanifra, Dialakoro et les villages environnants, un essai expérimental à été mis en place utilisant des isolats de terrain réactivés sur souris et inoculés à des veaux en étable sous moustiquaire. Ces veaux ont été suivis par BCT pendant 100 jours après le premier pic de parasitémie. L'essai expérimental n'a révélé aucun cas de rechute à 0,5mg/kg de poids vifs de chlorure d'isométymidium. De cette étude, il ressort que la chimiorésistance au chlorure d'isométymidium et à l'acéturate de diminazène, n'existe pas chez les bovins n'dama dans la zone cotonnière de la Haute Guinée.

**Mots clés:** *Trypanosoma congolense*, *Trypanosoma brucei*, bovins, isométymidium, résistance, Guinée.

## Summary

A survey on chemioresistance of trypanosomes in N'dama cattle was carried out in Upper Guinea. The study aimed to estimate the importance and distribution of chemioresistance in the Mandiana prefecture. For this purpose, a block treatment of isometamidium chloride (ISMM) at 1mg/kg bodyweight was carried out in the villages of Saladou, Kanifra, Dialakoro. Animals from the ISMM test group that were positives to BCT test, were treated with diminazene aceturate at 3,5mg/kg for those infected by *Trypanosoma congolense*, *T. vivax* and, at 7 mg/kg (*T. brucei*). A small proportion of animals varying from 2 to 6% were found to relapse during the longitudinal follow-up period of 56 days when animals were checked every fortnight by the buffy coat technique (BCT). No case of relapse for diminazene aceturate was observed. A second treatment was done in order to estimate the relapse around Saladou and Dialakoro villages. Ten and 5 villages were selected respectively around Saladou and Dialakoro. Three cases of trypanosomosis relapse were observed in 3 different villages around Saladou within the 28 days of follow-up of the animals. To specify the nature of the infections observed after the block treatments in Saladou, Kanifra, Dialakoro and the surrounding villages, an experiment was set up whereby the field isolates were reactivated in mice and then inoculated in calves housed in insect-proof stables. These calves were routinely examined by BCT during a period of 100 days after the first peak of the parasitaemia. The experimental test did not reveal any case of relapse to 0,5mg/kg of isometamidium chloride. From this study, it comes out that the chemioresistance against isometamidium chloride and diminazene aceturate, does not exist in N'dama cattle of the cotton belt of Upper Guinea.

**Keywords:** *Trypanosoma congolense*, *Trypanosoma brucei*, cattle, isometamidium, resistance, Guinea.

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## Introduction

En Afrique sub-saharienne, les trypanocides sont largement employés par les éleveurs. On estime en effet que 25 - 35 millions environ de doses de trypanocides sont administrées chaque année à approximativement 45 millions de bovins exposés au risque de la TAA (Kristjanson *et al.*, 1999). L'utilisation intensive de certains d'entre eux a amené l'apparition de résistance dans au moins 17 pays Africains (Delespaulx *et al.*, 2008). Au Burkina Faso, le phénomène de résistance a été observé (Authié, 1984 ; Clausen *et al.*, 1992) et s'étend au Mali (Diall *et al.*, 1992). Ces deux pays ont un système d'élevage similaire à celui appliqué dans la partie nord est de la Guinée.

En Guinée, précisément dans sa partie nord est (Mandiana), la trypanosomose affecte 3% des bovins. Les agro-éleveurs traitent leurs animaux soit avec le chlorure d'isométymidium ou l'acéturate de diminazène. Cet emploi de trypanocides préventif (chlorure d'isométymidium) et curatif (acéturate de diminazène) se fait sans discernement. Aucune information n'est disponible sur la chimiorésistance aux trypanocides dans la zone de Mandiana.

Le premier but de cette étude est de mettre en évidence en milieu naturel la sensibilité des populations de trypanosomes au chlorure d'isométymidium (ISMM) et à l'acéturate de diminazène. Le second est d'évaluer, sur des veaux maintenus en étable sous moustiquaire, les isolats de trypanosomes obtenus sur le terrain.

## Matériel et méthodes

### Zone d'étude

L'étude a été menée dans la préfecture de Mandiana. Elle est située à l'Est de la Guinée et présente une frontière avec le Mali et la Côte d'Ivoire. Sa superficie est de 12300 Km<sup>2</sup> (Figure 1).

Le climat est de type soudanien (moyennes pluviométriques annuelles de 1300 à 1500 mm et températures moyennes de 15° à 35°C) avec une saison des pluies de mai à octobre et une saison sèche de novembre à avril. Les bovins dans cette zone sont de la

race N'dama. Le type d'élevage est sédentaire et extensif. Les bovins sont quotidiennement conduits aux pâturages par un bouvier. Les mâles âgés de plus de 2 ans sont utilisés pour 4 heures/jour en traction durant la saison des pluies. Ils pâturent le reste de la journée et ont accès à l'eau le long des cours d'eau et au centre du village.

Les vecteurs *Glossina palpalis gambiensis* et *G. morsitans submorsitans* assurent la transmission de la maladie (Barry *et al.*, 2011).

### Période de l'étude :

L'étude s'est déroulée du 24 novembre 2002 au 19 janvier 2003 pour le traitement en bloc ;

- du 1<sup>er</sup> juin au 28 juin pour l'étude complémentaire utilisant un traitement en bloc ;
- du 1<sup>er</sup> mars 2004 au 8 juillet 2004 pour le test de détection de la chimiorésistance sur les veaux

### Traitement en bloc

Suite à l'étude transversale menée en octobre 2002 à Mandiana, trois villages ont été retenus (Saladou, Kanifra, Dialakoro) (Barry *et al.*, 2011). La sélection des villages a été faite en tenant compte de leur prévalence supérieure ou égale à 10% ou par la densité glossienne (Barry *et al.*, 2011).

Pour réaliser le traitement en bloc, cent animaux âgés de plus d'un an ont été retenus dans chacun des 3 villages soit 300 animaux.

Les animaux ont été bouclés, numérotés et prélevés à l'une des veines jugulaires, puis divisés en deux groupes de 50 animaux. Le groupe traité ou test (numéros impairs) et le groupe témoin (numéros pairs). Les animaux ont été suivis par BCT (Murray *et al.*, 1977). Le suivi a été fait chaque deuxième semaine pendant environ 2 mois (J0, J14, J28, J42, J56) sur les mêmes animaux.

Les animaux du groupe test ont été tous traités au chlorure d'isométymidium (SamorinR, Rhône Merieux, France) à raison de 1 mg/kg Poids vifs à J0. A J0 aucun animal du groupe témoin n'a reçu de traitement à l'isométymidium. Tous les animaux parasitologiquement positifs ont été traités à

l'aceturate de diminazène (BerenilR) à la dose de 3,5 mg/kg de poids vifs, exception faite aux animaux infectés par *Trypanosoma brucei* qui ont reçu la dose de 7 mg/kg de poids vif. Ce poids a été mesuré par un ruban barymétrique. Le bovin est debout dans une position normale. Le ruban est mis derrière les épaules pour prendre le tour de poitrine. La longueur en cm du tour de poitrine répondait à un poids qui était indiqué sur le verso du ruban.

Une seconde étude complémentaire par traitement en bloc, d'une durée de 28 jours a été réalisée dans 10 villages autour de Saladou et 5 à Dialakoro. L'objectif de la mise sur pied de ce second traitement en bloc a été de connaître dans un bref délai, le degré d'extension des échecs de traitement autour des villages où des rechutes ont été observées. C'est ainsi que dans chaque village, 80 bovins ont été identifiés par des boucles puis divisés en deux lots de 40 animaux. Le lot test a été protégé par une dose de 1mg/kg de poids vifs d'isométydium et le second a servi de témoin. Un suivi par BCT à J0, J14, J28 a été effectué. Les animaux positifs au BCT ont été traités au diminazène comme d'écrit ci-dessus.

*Essai expérimental sur des veaux N'dama maintenus en étable sous moustiquaire utilisant des isolats de terrain du traitement en bloc.*

L'essai a eu lieu au Centre International de la Trypanotolérance (ITC) de Banjul (Gambie).

#### *Les veaux*

Treize veaux de race N'dama, âgés de 1 an, de sexe mâle ont été utilisés pour l'essai expérimental. Les veaux ont été maintenus dans une étable sous moustiquaire, nourris aux fanes d'arachide séchées sans complément.

La répartition des veaux a été la suivante:

- Un contrôle positif pour chaque isolat,
- un groupe à traiter de trois veaux pour chaque isolat,
- un contrôle négatif ayant servi pour les trois isolats à la fois.

Un mois avant le test, les veaux ont été traités à l'albendazole à la dose de 0,75ml/kg de poids vifs afin de lutter contre les parasites

internes. Ils ont été traités par voie intramusculaire avec de l'aceturate de diminazène à la dose de 3,5mg/kg de poids vifs, puis traités par voie i.m à l'oxytétracycline longue action à la dose de 20 mg/kg de poids vifs. Les veaux ont été détiqués avec un « pour on » à base de deltaméthrine. A deux semaines de l'inoculation jusqu'à la fin de l'essai, un examen parasitologique par BCT (Murray *et al.*, 1977), trois fois par semaine, a été réalisé, suivi d'un examen clinique de l'animal. Les sérums des animaux ont été testés par l'ELISA détection d'anticorps de trypanosomes (Luckins, 1977) avant l'infection. Ces sérums se sont avérés négatifs.

Après confirmation de la vitalité des souches de trypanosomes par observation au microscope, les isolats ont été inoculés dans l'une des veines jugulaires de 12 veaux à raison de 105 trypanosomes/veau. Les veaux ainsi infectés ont été suivis parasitologiquement une fois par jour jusqu'au premier pic de parasitémie. Dès l'apparition de la parasitémie, 9 veaux ont été pesés à la balance électronique puis traités avec une solution d'isométydium à 2% à la dose de 0,5mg/kg de poids vifs. Les observations parasitologiques des veaux se sont poursuivies pendant 100 jours après le premier pic de parasitémie. L'hypothèse était que l'isolat soit déclaré sensible si l'animal infecté ne rechute pas dans un intervalle de 100 jours après le traitement (Eisler *et al.*, 2001).

#### *Les trypanosomes*

Au laboratoire de l'ITC, trois isolats ont pu être réactivés après une conservation en azote liquide : 2 isolats de l'espèce *T.brucei* (4165 GUI 05, 4235 GUI 05,) provenant des animaux du groupe traité de Saladou et 1 isolat de l'espèce *T.congolense* (5769 NJ) issu du groupe traité du village de Noumoudjila (village autour de Saladou). Le premier isolat du groupe traité (4165 GUI05) a été isolé d'un bovin ayant eu un BCT positif à J28. Le second de ce groupe (4235 GUI05) provenait d'un animal dont le BCT a été positif à J14. L'isolat 5769 NJ venait d'un bovin ayant eu un BCT positif à J28. Ces isolats de trypanosomes ont été confirmés par PCR (Clausen *et al.*, 1992 ; Clausen *et al.*, 1999) avant d'être utilisés pour assurer l'infection expérimentale des veaux.



Figure I : Sites de l'étude

Tableau I : Résultats de l'évaluation de l'efficacité de l'isométnamidium dans les villages de Saladou, Kanifra, Dialakoro.

Villages	Saladou		Kanifara		Dialakoro	
lots	Témoin	Traité	Témoin	Traité	Témoin	Traité
Bases / Villages	J0		J14		J28	
J0	1/50 (2%)Tv	0	1/50 (2%) Tb	2/50 (4%) Tb,TbTc	0	0
J14	2/49 (4%)Tc	3/50 (6%) Tc,Tv	2/49 (4%) Tb	2/48 (4%) Tb	2/50 (4%) Tb	3/50 (6%) Tb
J28	3/49(6%) Tc,Tv,Tb	2/50 (4%) Tv,Tc	0	1/48 (2%) Tb	0	0
J42	2/49(2%) Tc, Tv	3/50 (6%) TcTv	0	0	0	0
J56	1/49 (2%)Tc	1/50 (2%) Tc	0	0	0	0

\* 2 animaux absents Tv= Trypanosoma vivax ;Tc= Trypanosoma congolense ; Tb= Trypanosoma brucei

Le trypanocide

Le chlorure d'isométnamidium (SamorinR, Rhône Merieux, France) a été utilisé pour l'essai. Le sachet de 125 mg a été dilué dans un volume d'eau distillée de 6,25 ml soit une dilution de 2% pour l'injection en i.m des bovins de l'essai expérimental.

Résultats

Traitement en bloc au chlorure d'isométnamidium  
Lors du premier essai de traitement

en bloc par l'ISMM à 1mg/kg de poids vifs, le pourcentage d'animaux présentant une parasitémie, déterminée sur 300 animaux, 100 par village, était aux J0 de 1% (1/100) pour une infection à T. vivax à Saladou ; de 3% (3/100) pour les espèces T. congolense,T. brucei à Kanifra ; de 0% à Dialakoro (Tableau I). Les résultats des animaux du groupe test traité au chlorure d'isométnamidium ayant reçu du diminazène, dès l'expression d'une parasitémie aux trypanosomes dans leur sang, sont présentés dans le Tableau II. Pendant l'étude

**Tableau II:** Résultat de l'efficacité du diminazene chez les animaux du groupe test ayant reçu un traitement à l'aceturate de diminazène dès après un BCT positif dans les villages de Saladou, Kanifra, Dialakoro

Village	N° Animal	J0	J14	J28	J42	J56
<b>Saladou</b>	4165	0	0	Tv	Tc	0
	4183	0	0	0	Tc	0
	4207	0	0	Tc	0	0
	4225	0	0	0	TcTv	0
	4235	0	Tc	0	*	*
	4239	0	Tc	0	0	*
	4241	0	Tv	0	0	0
	4227	0	0	0	0	Tc
<b>Kanifra</b>	4409	Tb	0	0	0	0
	4443	0	Tb	0	0	0
	4469	0	Tb	0	0	0
	4481	TbTc	0	0	*	*
	4491	0	0	Tb	0	0
<b>Dialakoro</b>	4321	0	Tb	0	*	*
	4349	0	Tb	0	0	0
	4555	0	Tb	0	0	0

0 = animaux négatifs \* = animaux non retrouvés

**Tableau III :** Résultats de l'évaluation de l'efficacité de l'isométymidium dans les villages autour de Saladou et Dialakoro

		Traité	Contrôle	Traité	Contrôle	Traité	Contrôle
<b>Saladou</b>	Noumoudjila	2/40 Tc	4/40Tc	0	0	1/38*Tc	2/36Tc
	Wahiri	3/40 TvTc	1/40Tc	0	1/39Tv	0	0
	Tindila	3/40Tc	2/40Tc	0	3/38Tc	0	0
	Sèguèla	0	0	0	0	0	0
	Laminina	1/40Tc	0	0	0	0	0
	Ourala	0	0	0	0	0	1/40Tc
	Bakouna	0	0	1/40Tc	0	0	0
	Malétoumanina	0	0	1/40Tc	0	0	0
	Samakolen	0	0	0	0	1/40Tc	1/40Tc
	Linsoron	0	0	0	0	0	0
<b>Dialakoro</b>	Kantébalandou	1/40Tc	0	0	0	0	0
	Senké	2/40TcTv	0	0	0	0	0
	Faraba	0	0	0	0	0	0
	Farassababen	0	0	0	0	0	0
	Banafara	0	0	0	0	0	0

**Tableau IV :** Poids, PCV, Période pré patente, intervalle de rechute après traitement à l’ISMM des bovins N’dama infectés par 3 isolats de trypanosomes

Isolat	N° animal	Poids		PCV (Moy ± DS)	Période prépatente		Intervalle de rechute au traitement de 0,5 mg/kg PV
		début	fin		Jours	Moy ± DS	
4235 Gui05	22037*	99	104	22 ± 3,5	9	7 ± 2,6	-
	22030**	103	113	26 ± 3,8	5		-
	22013**	117	126	25 ± 3,1	5		-
	22029**	78	99	25 ± 3,3	10		-
4165 Gui05	22033*	125	127	20 ± 2,9	6	8 ± 3,2	-
	22035**	103	116	22 ± 2,7	12		-
	22032**	117	124	24 ± 2,8	11		-
	22036**	76	87	23 ± 2,0	6		-
5769 NJ	23004*	103	96	24 ± 2,5	9	7 ± 1,4	-
	22026**	112	130	24 ± 2,0	6		-
	22034**	87	103	24 ± 2,4	7		-
	22040**	62	64	20 ± 1,8	8		-
22038***		103	116	26 ± 1,7			

\* contrôle positif \*\* groupe traité \*\*\* contrôle négatif - pas de rechute

complémentaire du traitement en bloc par l’ISMM à 1mg/kg de poids vifs, le pourcentage d’animaux présentant une parasitémie, déterminée sur 1200 animaux, 80 par village, était aux J0 comme indiqué dans le Tableau III.

*Essai expérimental sur les veaux*

Après une observation de 100 jours des veaux, les infections avec les isolats 4165 Gui 05, 4235 Gui 05, 5769 NJ n’ont pas montré de rechute à la dose de 0,5 mg/kg de poids vifs. Les périodes pré patentes des infections variaient de 5 – 12 jours avec une moyenne de 7 - 8 jours (Tableau IV).

**Discussion**

L’étude qui porte sur les échecs de traitement sur le terrain a été conduite pour évaluer la résistance aux trypanocides des populations de trypanosomes qui circulent à Mandiana et préciser la nature de ces échecs dans la zone. Le pourcentage d’animaux qui rechute à l’ISMM semble faible de l’ordre de 2 à 6%. Ces proportions obtenues en dedans des 8 semaines après le traitement en bloc ne dépassent pas les 25%, ce qui est considéré comme seuil pour qu’on puisse parler de suspicion de résistance (Eisler et al., 2000).

Aucun animal des groupes test n’a présenté une parasitémie positive après un traitement à l’aceturate de diminazène. Les veaux ayant été inoculés par des isolats de terrain, n’ont pas rechutés après le traitement à l’ISMM. Ces différentes observations confirment que les trypanosomes sont sensibles au chlorure d’isoméтамidium et à l’aceturate de diminazène. Les cas de rechutes mis en évidence par le traitement en bloc, peuvent s’expliquer par le mauvais état des animaux. Les isolats de terrain provenaient d’animaux ayant un PCV en dessous de 20. Un bas PCV inférieur à 20, et un mauvais état du foie lors d’infestation à douves par exemple; réduisent particulièrement l’efficacité des trypanocides. La dowe est présente dans la zone d’étude. En effet, la séroprévalence de la fasciolose est de 47% en Haute Guinée (Barry et al., 2010).

L’étude complémentaire n’a révélé que 3 cas d’infection dans 3 villages différents sur les 15 observés, uniquement autour de Saladou. Lorsqu’on traite un groupe d’individus, il est possible qu’il y en ait un qui ne réponde pas au traitement. Cela semble être lié à l’individu mais pas à la sensibilité de la population de trypanosomes.

## Conclusion

La chimiorésistance en trypanocides chez les bovins n'dama n'existe pas dans la zone cotonnière de la Haute Guinée.

L'étude a trouvé que les isolats de terrain testés in vivo sur des veaux sont sensibles au chlorure d'isométylmidium à demi dose 0,5 mg/kg de Poids vifs. Il convient d'évaluer sur le terrain, cette demi-dose sur bovin N'dama pour minimiser le risque d'apparition de la résistance dans la zone.

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## SEROLOGICAL SURVEY OF BRUCELLOSIS IN FOOD ANIMALS IN OGUN STATE, NIGERIA

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### Abstract

A serological survey of brucellosis in food animals was conducted in Abeokuta, the largest city in Ogun State, Nigeria between August and November 2009. A total of 275 cattle, 52 sheep, 31 goats and 30 pigs were screened for Brucellosis using Rose Bengal Plate Test (RBPT). The Standard Tube Agglutination Test (STAT) was carried out on sera samples that were positive for brucellosis to quantitatively determine the level of antibody titre. Brucellosis was confirmed in 14.2%, 9.6% and 12.9% of cattle, sheep and goat respectively. All 30 pigs were negative for brucellosis. All sheep and goats were positive at 1:10 dilution, while 10.3%, 38.5% and 51.3% of positive cattle were positive at 1:10, 1:20 and 1:40 dilutions respectively. This result showed that brucellosis is still an important infectious disease of food animals and possibly a major zoonosis in Ogun State, Nigeria.

**Keywords:** Rose Bengal Plate Test, Standard Tube Agglutination Test, Brucellosis, Nigeria

## ENQUETE SEROLOGIQUE SUR LA BRUCELLOSE CHES LES ANIMAUX DESTINES A L'ALIMENTATION HUMAINE DANS L'ETAT D'OGUN AU NIGERIA

### Résumé

Une étude sérologique de la brucellose chez les animaux destinés à l'alimentation humaine a été menée à Abeokuta, la plus grande ville de l'État d'Ogun au Nigeria, entre août et novembre 2009. Au total, 275 bovins, 52 moutons, 31 chèvres et 30 porcs ont été testés en vue de rechercher la présence de la brucellose en utilisant l'épreuve sur lame au rose bengale (RB). Le test d'agglutination en tube standard (STAT) a été réalisé sur des échantillons de sérums positifs pour la brucellose, afin de déterminer quantitativement le niveau du titre des anticorps. La brucellose a été confirmée respectivement chez 14,2%, 9,6% et 12,9% des bovins, moutons et chèvres. Tous les 30 porcs étaient négatifs pour la brucellose. De cet ensemble d'animaux, tous les moutons et chèvres étaient positifs à la dilution de 1:10, tandis que 10,3%, 38,5% et 51,3% de bovins positifs étaient positifs respectivement aux dilutions de 1:10, 1:20 et 1:40. Ce résultat a montré que la brucellose reste une maladie infectieuse importante des animaux destinés à l'alimentation humaine et éventuellement une zoonose majeure dans l'État d'Ogun au Nigeria.

**Mots-clés :** Epreuve sur lame au rose bengale ; Test d'agglutination en tube standard ; Brucellose ; Nigeria

## Introduction

Brucellosis is a chronic disease of animals caused by Gram negative and facultative non-motile intracellular bacteria of the genus *Brucella* and is characterized by abortion, retained placenta and impaired fertility in females and to a lesser extent, orchitis and infection of the accessory sex glands in males. It is one of the most important zoonotic diseases worldwide, and is of particular significance in developing countries. Brucellosis is also called Undulant fever, Mediterranean fever, Malta fever, and Bang's disease. Sheep and goats and their products remain the main source of infection, and in some areas, cattle now more important than pigs as a source of human infection. Contamination can be by ingestion, inhalation or contact with conjunctiva or traumatised skin by infected animal products (Dobrea et al., 2002).

Brucellosis was first reported in Nigeria in 1927 as published in the animal reports of the Veterinary Department for that year in which ten cases of contagious abortion were reported (Anon, 1927). Evidence of the presence of *Brucella* infection has been demonstrated in livestock and humans in Nigeria (Ocholi et al., 1993). Studies confirming the presence of brucellosis in livestock have been documented by several workers (Adamu and Ajogi, 1999; Ajogi et al., 1998; Brisibe et al., 1993; Cadmus et al., 2006; Esuruoso, 1974; Falade et al., 1975; Ishola and Ogundipe, 2001; Ogundipe et al., 1994; Oyekunle et al., 2007) thereby demonstrating the endemicity of brucellosis in Nigeria.

One of the most common serological tests used for the screening and diagnosis of brucellosis is the Rose Bengal Plate Test (RBPT). The test is based on the agglutination of coloured particulate antigen (killed *Brucella* organisms) by the antibodies present in the test serum. Although it is a simple, cheap and effective test, the RBPT is generally considered to be less sensitive than other tests like standard tube agglutination test (STAT), complement fixation test (CFT) and Enzyme Linked Immunosorbent Assay (ELISA) (Chachra et al., 2009). The STAT method has been utilized in the study of the agglutinins present

in the sera of humans and animals infected with *B. canis* (Damp et al., 1973). The Standard Tube Agglutination Test was developed to yield results using a minimal amount of equipment, time, reagents and serum. The zoonotic importance of brucellosis necessitates regular surveillance using economical but effective screening techniques to detect carrier status in animals.

This study was conducted to determine the presence of brucellosis in cattle, sheep, goats and pigs in Abeokuta, South-Western Nigeria, using a combination of two sero-diagnostic tests, RBPT and STAT.

## Materials and Methods

### Location of study

The study was conducted in Abeokuta, Ogun State, South west Nigeria (around 7° 9' 39" N, 3° 20' 54" E) where blood samples were collected from 275 cattle, 52 sheep, 31 goats and 30 pigs and screened over a period of four months between August and November, 2009.

### Sample collection

Blood samples were collected aseptically from the jugular vein of cattle, sheep and goat, and the anterior vena cava of pigs using sterile syringes. This was transferred into sterile labelled sample bottles containing no anticoagulant, placed in slanted positions to assist in serum separation and transported to the Microbiology laboratory of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. Blood samples were allowed to clot for 3 hours at room temperature, after which sera were decanted into test tubes and centrifuged at 1,500g for 10 minutes and thereafter decanted into labelled bijou bottles and stored at -4°C until use.

Rose Bengal Plate Test (RBPT): All sera samples were screened using the RBPT as earlier described (Morgan et al., 1969). Ten microlitre of RBPT antigen (Bio Onderstepoort) was mixed with an equal volume of test serum on a clean grease-free glass slide using a sterile inoculating loop. The reaction was examined after 1-2 minutes for the presence of agglutination. Sera samples that produced agglutination with the RBPT antigen

were considered positive for *Brucella* antibody while those that failed to produce agglutination were considered negative.

**Standard Tube Agglutination Test (STAT):** Sera samples that were positive for brucellosis by RBPT were further subjected to STAT for the quantitative determination of antibody titre as previously described (Bertu *et al.*, 2010). The method involved the use of five test tubes per sample. In the 1st tube, 0.8ml of phenol saline was dispensed while 0.5ml was applied to the 2nd, 3rd, 4th and 5th tubes using microtitre pipette fitted with corresponding tips. Similarly, 0.2ml of the test serum was added to the 1st tube and mixed properly. Serial dilution was then carried out by pipetting 0.5ml of mixture in the 1st tube to 2nd, then to the 3rd, then to the 4th and then the 5th tubes. The final 0.5 ml from the 5th tube was discarded. 0.5ml of antigen (diluted 1:10 with phenol saline) was added to all the tubes. The tubes were covered, shaken and incubated at 37°C for 20 hours. The result was read, agglutination titres determined and interpreted according to the recommendation of the FAO/WHO Expert Committee on brucellosis as follows: Titres of 1:40 (50 IU/ml at 1:40) or above were taken as diagnostic for brucellosis, 1:20 was taken as suspicious while 1:10 was taken as negative (Alton *et al.*, 1975; Ogunidipe *et al.*, 1994b). Positive and negative control sera were also set up along the experiment.

## Results

In the present study, 48 (12.4%) of all 388 sera samples examined were positive for brucellosis as determined by RBPT. The prevalence of brucellosis was higher in cattle (14.2%) than in goats (12.9%) and sheep (9.6%) while all the sampled pigs were negative.

Of the 48 sera samples positive by RBPT, 20 (5.2%) were further confirmed to be positive by STAT with antibody titre of 1:40 and above, 15 (3.9%) were considered suspicious with antibody titre of 1:20 while 13 (3.4 %) were negative with antibody titre of 1:10 and below (Table 1).

## Discussion

This study highlights the significance of using more than one serological test in screening for brucellosis. The RBPT was developed many years ago for the diagnosis of bovine brucellosis (Morgan *et al.*, 1969) and has also been internationally recommended for the screening of the disease in small ruminants (Garin-Bastuji and Blasco, 1997). Although RBPT is rapid, simple and sensitive, it is of low specificity (Flad, 1983) and some commercial RB antigens are of relatively low sensitivity (Blasco *et al.*, 1994). Nevertheless, RBPT is reliable in giving a high degree of reproducibility and accuracy.

Although no single serological test can be said to provide 100% sensitivity and specificity, the RBPT was adopted because of its high sensitivity (99%) (Nielsen, 2002). Since the positive predictive value of this test is low, a positive result must to be confirmed by other more specific test like STAT. On the other hand, STAT is the most frequently used confirmatory serological test and has become the standard method for the diagnosis of brucellosis. The sensitivity and specificity of STAT are reported to be 95.6% and 100%, respectively (Memish *et al.*, 2002). The high *Brucella* agglutinating antibody titre of 1:40 observed in the present STAT study indicated active *Brucella* infections in cattle while titres of 1:20 or less may be due to previous infection or subclinical exposure to *Brucella* (Alton *et al.*, 1975).

This study showed a prevalence of 5.2% positive sera (active brucellosis) by STAT on RBPT positive samples, which is significant and considerable from the perspective of public health. The prevalence of brucellosis in cattle in the present study is similar to the 18.3% reported in cattle and buffaloes at organized private farms (Nasir *et al.*, 1999). However, a lower prevalence of 5.8% was reported in another study (Cadmus *et al.*, 2006). The continuous presence of the brucellosis (especially active brucellosis) depicts the endemicity of this disease in cattle in Nigeria. It also suggests that *Brucella* may continually be shed by infected animals hereby constituting public health risk to people in high-risk occupations such as veterinarians, farmers,

**Table 1:** Prevalence of Brucellosis and antibody titre in food animals in Ogun State, Nigeria

Species	No of animals samples tested	No of positive sera	No of animals with antibody titre		
			1:10	1:20	1:40
Cattle	275	39 (14.2%)	4	15	20
Goats	31	4 (12.9%)	4	0	0
Sheep	52	5 (9.6%)	5	0	0
Pigs	30	0 (0.0%)	0	0	0
Total	388	48 (12.4%)	13	15	20

meat handlers, abattoir workers and animal traders. *Brucella* pathogens can be transmitted to humans through direct contact with raw meat and carcasses of infected animals, cuts and wounds, or through splashing of infected blood or other fluid to the conjunctiva (Madkour and Gargani, 1985).

The transportation and continuous introduction of unscreened trade animals to central cattle markets in South-West Nigeria from different sources extending from neighbouring countries like Niger, Chad, Burkina Faso and Cameroon down to the northern regions of Nigeria further increases the chances of spread of the disease among animals and puts humans at risk.

**Conclusion**

This study demonstrated the presence of *Brucella* infections in cattle in Ogun State, Nigeria and hence, brucellosis is still an important infectious disease of food animals and possibly a major zoonosis in this area. Animal handlers at all levels are exposed to the risk of zoonotic transmission of the disease. The risk of human infection posed by the continuous presence of brucellosis can only be limited by improved hygiene and proper screening of animals before they are slaughtered at the abattoir. The screening can be carried out using RBPT and STAT which are complementary to each other, cheap, easy to perform, do not require sophisticated facilities and therefore suitable in developing countries.

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**Impact**

A serological survey of brucellosis in food animals conducted in Abeokuta, Nigeria between August and November 2009 indicated that Brucellosis was confirmed in 14.2%, 9.6% and 12.9% of cattle, sheep and goat respectively. Of these, all sheep and goats were positive at 1:10 dilution, while 10.3%, 38.5% and 51.3% of positive cattle were positive at 1:10, 1:20 and 1:40 dilutions respectively. This result showed that brucellosis is still an important infectious disease of food animals and possibly a major zoonosis in Ogun State, Nigeria.

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## CLINICAL OBSERVATIONS ON THREE NIGERIAN ZEBU CATTLE BREEDS FOLLOWING EXPERIMENTAL *TRYPANOSOMA CONGOLENSE* INFECTION

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### Abstract

Three yearling cattle of each of three major Nigerian zebu breeds, viz: White Fulani (WF), Red Bororo (RB) and Sokoto Gudali (SG) were intravenously infected with  $1.5 \times 10^6$  *Trypanosoma congolense* and monitored until the PCV declined to  $\leq 15\%$ , when they were treated with diminazene aceturate. Two cattle of each breed served as uninfected controls. Parasitaemia, which ranged between  $1.0 \times 10^3$  and  $5.0 \times 10^5$  trypanosomes/ml, was significantly ( $P < 0.05$ ) higher in SG and RB than in their WF counterpart. Signs of trypanosomosis became apparent before detection of parasitaemia, 7 to 10 days post infection (pi) firstly in SG, then RB and lastly in WF. The infected animals were dull, lagged behind the herd, had depressed appetite and pale mucous membranes. The live weight gains of infected cattle were significantly ( $P < 0.05$ ) lower than those of non-infected cattle. Live weight loss was least in WF (5.2%), intermediate in RB (6.5%) and highest in SG (10.3%) at 35 days pi. Of the three infected SG, two died at a mean of  $28.0 \pm 14.1$  days pi, while the third was treated 38 days pi. Infected RB and WF were treated at a mean of  $39.3 \pm 2.3$  and  $54.7 \pm 2.3$  days pi, respectively. No infected RB or WF died before chemotherapy. It was concluded that of the three cattle breeds studied, the WF was the least susceptible to trypanosomosis, while SG was the most susceptible.

**Keywords:** Zebu cattle, Susceptibility, *Trypanosoma congolense*, Clinical signs

## OBSERVATIONS CLINIQUES DE TROIS RACES DE BOVINS ZEBUS NIGERIENS APRES UNE INFECTION EXPERIMENTALE AVEC *TRYPANOSOMA CONGOLENSE*

### Résumé

Trois bovins âgés d'un an, sélectionnés chacun dans les trois grandes races de zébus nigériens, à savoir Fulani blanc (FB), Bororo rouge (BR) et Sokoto Goudali (SG), ont été infectés par voie intraveineuse avec *Trypanosoma congolense* à raison de  $1,5 \times 10^6$  et surveillés jusqu'à ce que la valeur d'hématocrite ait diminué à  $\leq 15\%$ , et ils ont ensuite été traités avec l'acéturate de diminazène. Deux bovins de chaque race ont été utilisés comme témoins non infectés. La parasitémie, qui variait entre  $1,0 \times 10^3$  et  $5,0 \times 10^5$  trypanosomes / ml, était significativement ( $P < 0,05$ ) plus élevée chez les races SG et BR par rapport à la race FB. Les signes de trypanosomose sont devenus évidents avant la détection de la parasitémie, 7 à 10 jours post-infection (pi) d'abord chez les SG, puis chez les BR et enfin chez les FB. Les animaux infectés étaient faibles, traînaient derrière le troupeau, avaient peu d'appétit, et leurs muqueuses étaient pâles. Les gains de poids vifs des bovins infectés étaient significativement ( $P < 0,05$ ) inférieurs à ceux des bovins non-infectés. La perte de poids vif était moindre chez les FB (5,2%), intermédiaire chez les BR (6,5%) et plus élevée chez les SG (10,3%) à 35 jours pi. Des trois SG infectés, deux sont morts à une moyenne de  $28,0 \pm 14,1$  jours pi, tandis que le troisième a été traité 38 jours pi. Les BR et FB infectés ont été traités respectivement à une moyenne de  $39,3 \pm 2,3$  et de  $54,7 \pm 2,3$  jours pi. Aucun bovin BR ou FB n'est mort avant la chimiothérapie. Il a été conclu que des trois races bovines étudiées, la race FB était la moins sensible à la trypanosomose tandis que la race SG était la plus vulnérable à la maladie.

**Mots-clés :** Bovins zébus ; Sensibilité ; *Trypanosoma congolense* ; Signes cliniques

## Introduction

African animal trypanosomosis (AAT) has greatly hampered human settlement and economic development in vast areas of sub-Saharan Africa (Lutje *et al.*, 1996). The disease complex is caused by tsetse-fly-transmitted *T. congolense*, *T. vivax* or *T. brucei* or concurrent infection with two or more of these trypanosomes (Mare, 1998). An infection with one of these trypanosome species may result in a chronic, debilitating, emaciating and often fatal disease but the outcome of the infection differs substantially between trypanosome species, between livestock species and among breeds within a livestock species (Masumu *et al.*, 2006.).

*Trypanosoma congolense* and *T. vivax* are generally considered to be more pathogenic to cattle, as assessed by their capacity to induce anaemia, depress live weight and, eventually, cause death than *T. brucei* (Mattioli *et al.*, 1999). In West Africa, *T. congolense* is known to be more commonly associated with the chronic form of the disease while *T. vivax* predominates in the acute form (Stephen, 1986).

In regions of Africa infested with tsetse flies, increasing consideration is being given to selection and propagation of breeds of livestock with the innate ability to withstand the effects of trypanosome infections (Katunguka-Rwakishaya *et al.*, 1997). Trypanotolerance, the ability of some breeds of several mammalian species (such as cattle, sheep, goats, pigs, wild buffaloes and antelopes) to live normally and remain productive in tsetse-infested areas (Berthier *et al.*, 2003), was recognized and exploited by farmers long before formal research on trypanotolerance began (d'Ieteren and Kimani, 2001). Trypanotolerant cattle are small and humpless breeds that include the N'dama and the West African short-horned cattle, which, depending on the region where it is found, are variously called Muturu, Baole, Laguna, Samba and Dahomey (Dolan, 1987). The N'dama is the most important cattle breed expressing the trypanotolerant trait (Trail *et al.*, 1993) but can succumb to the disease under stress (Otesile *et al.*, 1991). Susceptibility studies have shown the N'dama to be the most resistant breed, followed by the smaller West

African short-horned cattle, while the large zebu is the most susceptible (Mare, 1998).

The estimated population of N'dama cattle in Nigeria is 15,000 (RIM, 1993), accounting for about 0.11% of the Nigerian cattle population. The vast majority of Nigerian cattle are zebu breeds. The zebu breeds of cattle are varied in their susceptibility to trypanosomosis as demonstrated amongst East African zebu cattle, where the Maasai zebu and the Orma Boran had superior tolerance over the Galana Boran (Mwangi *et al.*, 1998). In Nigeria, there is a dearth of information on the relative susceptibility of the various zebu cattle breeds to trypanosomosis (Talabi, 2006). This paper presents the clinical observations including the changes in live weight gain of three major Nigerian zebu breeds, viz: White Fulani (WF), Red Bororo (RB) and Sokoto Gudali (SG) following experimental infection with *Trypanosoma congolense*. In the West African sub-region, the three breeds are also referred to as Bunaji (WF), Rahaji (RB) and Bokolo (SG).

## Materials and Methods

### Location of Study

This study was carried out in the Faculty of Veterinary Medicine of the University of Ibadan, Nigeria, an area of virtually no tsetse challenge.

### Experimental animals

Fifteen yearling bulls, 12 to 15 months of age, made up of five animals from each of three indigenous Nigerian zebu cattle breeds, viz: White Fulani, Red Bororo and Sokoto Gudali, were obtained from Talata Mafara, Zamfara State, a tsetse fly free area of Nigeria. The animals were ear tagged, sprayed with Diazinon dimpylate (Diazintol(R), Alfasan International B.W. Holland) for the control of ectoparasites and screened for blood and faecal parasites. The following medications were given 4 days after the arrival of the animals: Intramuscular injection of diminazene aceturate (Samorenil(R), Alfasan International B.W., Holland) at 7mg/kg live body weight, intramuscular injection of long acting oxytetracycline (Oxitetraciclina 200 LA(R), Invesa, Spain) at 20mg/kg live body weight and albendazole bolus (Sambezole(R),

Sam Pharmaceuticals, Nigeria) at 8.3mg/kg body weight.

#### *Animal management*

The bulls were allowed 8 hours of grazing time per day while supplementary feeding was provided in a paddock where the animals were kept overnight. The supplementary feed consisted of one part dried cassava peels to one part of guinea corn offal. Salt lick and water were provided *ad libitum*. The 15 animals were protected from flies and ticks throughout the course of the study using cypermethrin pour-on every two weeks. The animals were stabilized for three months before challenge with *Trypanosoma congolense*.

#### *Trypanosome stock*

A stock of *T. congolense* isolated from cattle in Kaura, Kaduna State in 1995 was obtained from the Nigerian Institute for Trypanosomosis Research (NITR), Vom, Plateau State, Nigeria and passaged into mice. To expand the isolate, the mice were bled through the retro-orbital plexus and an adult male Sahel Brown goat, weighing 11.5kg was infected with  $1.0 \times 10^5$  *T. congolense* organisms through the jugular vein. The goat became parasitaemic 8 days post-infection (pi) and on 14 days pi, when the parasitaemia was high, the zebu cattle were infected.

#### *Experimental design*

Three animals of each breed were infected with 3ml of goat blood containing  $0.5 \times 10^6$  *Trypanosoma congolense* per ml through the jugular vein. Two animals of each of the three breeds served as the uninfected control cattle.

#### *Sample collection*

Prior to infection of the animals, 6ml of blood was collected from the jugular vein of each of the 15 zebu cattle; 2ml into sterile bijoux bottles containing 100µl of 200mM of Disodium ethylenediamine tetra-acetic acid ( $\text{Na}_2\text{EDTA}$ ) solution, and the remaining 4ml into sterile plain test tubes for serum production. After infection, all animals were sampled twice weekly.

#### *Estimation of live weight.*

The live weight of all the animals were measured weekly throughout the course of this experiment using a 0.5 tonne Avery platform scale.

#### *Estimation of parasitaemia*

The number of trypanosomes in each of the blood samples was estimated using the buffy coat parasitaemia scoring system as described by Paris *et al.*, (1982). Briefly, the capillary tube used for the measurement of the PCV was cut about 1mm below the buffy coat and the contents of the coat expressed on to a clean microscope slide. This was mixed with a small quantity of supernate plasma, covered with a microscope cover slip and examined under the light microscope at x400 magnification.

#### *Treatment of severe infection*

To forestall unnecessary suffering and death of infected animals, cattle were treated with diminazene aceturate at a dosage of 7mg/kg whenever the PCV declined to 15% or less.

#### *Post mortem Examination of Dead cattle*

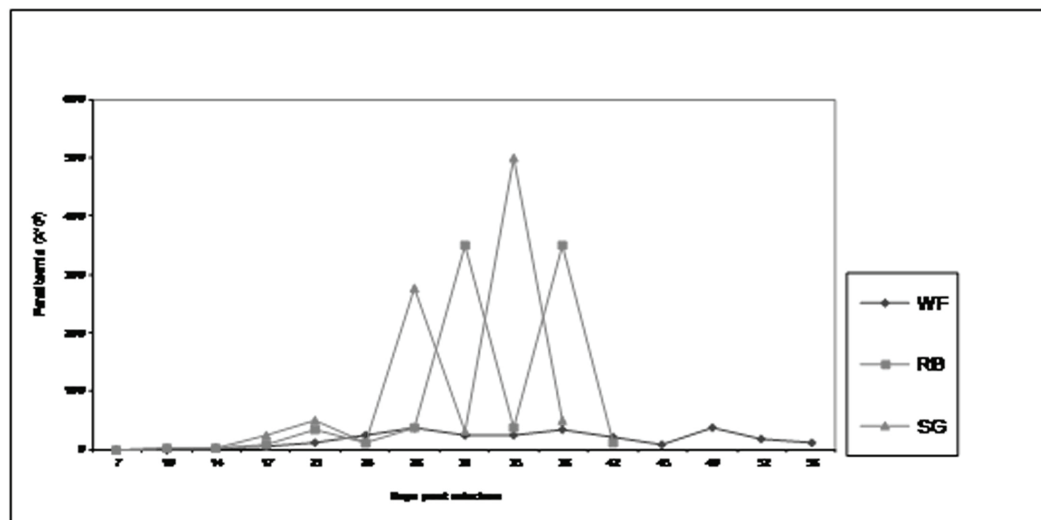
Post mortem examination was carried out on all dead animals.

#### *Statistical analysis*

Data were analysed using two-way analysis of variance (ANOVA) using the SAS (1999) software package. Associations between parameters were quantified with the Duncan's multiple range tests.

## **Results**

Development of parasitaemia was detected in infected SG, RB and WF at a mean of  $11.3 \pm 2.3$ ,  $12.7 \pm 2.3$  and  $14.0 \pm 0.0$  days pi, respectively. The parasitaemia, which ranged between  $1.0 \times 10^3$  and  $5.0 \times 10^5$  trypanosomes per ml of blood, was significantly ( $P < 0.05$ ) higher in SG and RB than their WF counterpart throughout the study (Figure 1). No parasites were detected in any of the non-infected controls throughout the duration of the experimental study.



**Figure 1:** Mean parasitaemia ( $\times 10^3$ ) of cattle infected with *T. congolense*

Signs of trypanosomosis became apparent first in SG, then RB and WF, 7 to 10 days pi. While the uninfected control animals were bright, alert with smooth and shiny coat and moist muzzle, the opposite was the case for the infected cattle. In this early stage (between 7 and 15 days pi in the Red Bororo and Sokoto Gudali and between 10 and 33 days pi in the White Fulani) they were dull, lagged behind the herd, often sought shade and stood idle. There was depressed appetite, progressive loss of condition, weakness, weight loss and enlargement of the lymph nodes.

At the latter stage of the infection (between 16 and 28 days pi in the RB and SG, and between 34 and 46 days pi in the WF), infection was more severe with mucous membranes being pale, while jugular pulsation, salivation, nasal discharge and lacrimation were seen. Blood became watery, while dehydration, emaciation and loss of condition were noticed. Sub-mandibular oedema was observed in a Red Bororo. Infected animals became recumbent early in the morning and had to be helped to rise to join others to graze. Terminally, they lay quietly in lateral recumbency, show no central nervous system involvement and died.

The live weight gains of non-infected and *T. congolense* infected cattle are presented in Figure 2. The live weight gains of infected cattle were significantly ( $P < 0.05$ ) lower than those of non-infected cattle as from 14 days pi in the RB and SG, and as from 21 days pi

in WF cattle. By 35 days pi, live weight loss was lowest in WF (5.2%), intermediate in RB (6.5%) and highest in SG (10.3%). Uninfected control animals maintained a fairly consistent growth rate until the end of the study. Mean weight gains were 11.5%, 8.6% and 6.2% for the uninfected control WF, RB and SG cattle, respectively.

Two Sokoto Gudali cattle died at a mean of  $28.0 \pm 14.1$  days pi while the third was treated 38 days pi with diminazene aceturate. Red Bororo cattle were treated at a mean of  $39.3 \pm 2.3$  days pi (38, 38 and 42 days pi). White Fulani cattle were treated at a mean of  $54.7 \pm 2.3$  days pi (52, 56 and 56 days pi). At necropsy, carcasses were pale with general loss of condition. The viscera were pale, the spleen, lymph nodes and heart were enlarged, while excessive serous fluid was found in the pericardium, thoracic and abdominal cavities. The bone marrow showed extensions of the red haemopoietic areas into the medullary cavity of long bones.

## Discussion

Control of parasitaemia is considered as an indicator of resistance to trypanosome infection (Paling *et al.*, 1991). In this study, the parasitaemia was significantly ( $P < 0.05$ ) higher in SG and RB than their WF counterpart throughout the study. Also, WF had the longest incubation period thus providing further

evidence that in cattle, the ability to control parasitaemia results in delayed onset of clinical signs. Therefore, among different breeds of cattle, the time taken for appearance of clinical signs and control parasitaemia can be recommended as criteria for evaluating relative susceptibility to trypanosomosis.

Trypanotolerance has been defined as the relative capacity of an animal to control the development of the parasites and to limit their harmful effects, the most prominent of which is anaemia (Verhulst and Pandey, 1992). Results in recent years have shown that these two parameters are strongly correlated with animals performance, especially post-weaning growth, reproductive performance and overall cow productivity (d'Ieteren and Kimani, 2001).

The live weight gains of infected cattle were significantly ( $P < 0.05$ ) lower than those of non-infected cattle as from 14 days pi in the RB and SG, and as from 21 days pi in WF cattle. Live weight loss was lowest in WF, intermediate in RB and greatest in SG at 35 days pi.

Associated with the ability to control *T. congolense* challenge, WF had the least weight losses. Results from this study provided further evidence that in cattle, the ability to control parasitaemia and limit trypanosome-induced anaemia results in superior weight gain. Therefore, among cattle breeds, anaemia and ability to minimize weight loss can be recommended as reliable criteria to evaluate the level of trypanotolerance.

Infected animals were dull, lagged behind the herd, often sought shade and stood idle. At the severe stage of the infection (between day 16 and 28 in RB and SG, and between day 34 and 46 in WF), mucous membranes were pale, jugular pulsation and lacrimation were seen, blood was watery, while dehydration, emaciation and loss of condition were noticed. Sub-mandibular oedema was observed in a Red Bororo. Infected animals became recumbent early in the morning and had to be helped to rise and join others for grazeing. These findings are in agreement with the observations made by Mare (1998) and (Uilenberg and Boyt, 1999).

Of the three infected SG, two died at a mean of  $28.0 \pm 14.1$  days pi, while the third was treated 38 days pi. Infected RB and WF were treated at a mean of  $39.3 \pm 2.3$  and  $54.7 \pm 2.3$

days pi, respectively. Together with other clinical findings, this observation indicated that the SG was the most susceptible breed to trypanosomosis followed by the RB, while the WF was the least susceptible. Even though no pathognomonic change is seen at necropsy in trypanosomosis (Mare, 1998), the changes observed in this study indicated that death was due to severe anaemia.

In conclusion, the three Nigerian zebu cattle breeds used in this study showed variations in their susceptibility to *Trypanosoma congolense*: Sokoto Gudali (Bokolo) cattle was the most susceptible, Red Bororo (Rahaji) intermediate, while White Fulani (Bunaji) was the least susceptible.

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### Impact

Three major Nigerian zebu breeds, viz: White Fulani (WF), Red Bororo (RB) and Sokoto Gudali (SG) were intravenously infected with  $1.5 \times 10^6$  *Trypanosoma congolense* and monitored until the PCV declined to  $\leq 15\%$ , when they were treated with diminazene aceturate. Parasitaemia, signs of trypanosomosis, live weight gains showed that the WF was the least susceptible to trypanosomosis, while SG was the most susceptible.

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## ENQUETE SEROLOGIQUE SUR LES MYCOPLASMOSES AVIAIRES CHEZ LES POULES PONDEUSES DANS LES ELEVAGES AVICOLES AMELIORES EN ZONES PERI-URBAINES DU MALI

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### Résumé

Une enquête sérologique a été menée pour déterminer la prévalence d'anticorps contre les mycoplasmoses aviaires chez les poules pondeuses dans les fermes avicoles améliorées en zone périurbaine des villes de Sikasso, de Ségou et du District de Bamako au Mali. Au total 360 sérums ont été prélevés et analysés par le test d'ELISA indirect pour la recherche d'anticorps contre *Mycoplasma synoviae* (MS) et *Mycoplasma gallisepticum* (MG). Les résultats obtenus indiquent un taux global de prévalence de 67,50 % (243/360) pour MS et de 31,39 % (113/360) pour MG. Le District de Bamako a enregistré les plus forts taux de prévalence avec respectivement 87,80 % et 41,46 %; tandis que pour Sikasso, ces taux ont été respectivement de 51,72 % et 32,76 % et pour Ségou de 71,43 % et 7,94 % respectivement. Puisqu'à l'heure actuelle, la vaccination contre les mycoplasmoses chez la volaille n'est pas pratiquée au Mali, ces résultats reflètent certainement l'infection et montrent que ces pathologies pourraient être cliniquement importantes dans les fermes avicoles. D'où la nécessité d'effectuer des sondages microbiologiques afin d'établir une épidémiologie plus précise.

**Mots-clés:** Mycoplasmoses aviaires, fermes avicoles, prévalence sérologique, Mali

## SEROLOGICAL SURVEY OF AVIAN MYCOPLASMOSES AMONG LAYER HENS IN THE SEMI-INTENSIVE POULTRY FARMS IN PERI-URBAN AREAS OF MALI

### Summary

A serological survey was conducted to determine the prevalence of antibodies against avian mycoplasmoses among layer hens in the semi-intensive poultry farms in peri-urban areas of Sikasso, Ségou and the District of Bamako in Mali. A total of 360 sera were collected and analyzed by indirect ELISA test for the detection of antibodies against *Mycoplasma synoviae* (MS) and *Mycoplasma gallisepticum* (MG). Results showed an overall seroprevalence rate of 67.50 % (243/360) for MS and 31.39 % (113/360) for MG. The District of Bamako has recorded the highest prevalence rates respectively with 87.80 % and 41.46 %. While for Sikasso, these rates were respectively of 51.72 % and 32.76 % and for Ségou they were 71.43 % and 7.94 %, respectively. Since vaccination against avian mycoplasmoses is not performed in Mali, these results certainly reflect the presence of the infection and indicate that these pathologies may be clinically important in poultry farms in Mali. Hence the need to carry out microbiological surveys to isolate the *Mycoplasma* species involved, so that the epidemiology can be specified accurately.

**Keywords:** Avian mycoplasmoses, poultry farms, seroprevalence, Mali.

## Introduction

Le cheptel aviaire au Mali est estimé à 26 millions de sujets toutes espèces confondues (DNSV, 2008). Il se répartit entre un secteur traditionnel disséminé sur l'ensemble du territoire national (95 à 98 % des sujets) et un secteur amélioré concentré autour des grandes villes du pays (2 à 5 % des sujets) (DNPIA, 2008). Ce dernier, en plein essor, a pour vocation de satisfaire les besoins des populations urbaines en œufs et en poulet de chair. Du fait de l'existence d'un marché en expansion, ce secteur attire de nombreux opérateurs urbains (hommes d'affaires, groupements féminins, jeunes diplômés, fonctionnaires), tous soucieux de rentabiliser une activité réputée consommatrice d'intrants.

Cependant, bien qu'étant une activité en plein essor, cette aviculture présente de contraintes de productions très accrues. En effet, le taux de ponte des pondeuses dans ces élevages est de 60 % en moyenne et le poids moyen de carcasse pour le poulet de chair est de 1,700 kg (DNPIA, 2008). Cette faible productivité est liée essentiellement à la non maîtrise de certains facteurs tels que l'alimentation et la santé.

Les plus en vue parmi ces contraintes sanitaires sont la maladie de Newcastle, la maladie de Gumboro, les salmonelloses et les colibacilloses, essentiellement en raison des taux élevés de mortalité qui leur sont attribués (LCV, 2009; Sylla *et al.*, 2003; Tounkara *et al.*, 1995). D'autres maladies plus discrètes dans leur évolution, comme les mycoplasmoses, sont rarement prises en compte par les éleveurs et les cliniciens. Cependant, celles-ci peuvent causer des pertes économiques très importantes se traduisant par des retards de croissance, une augmentation de l'indice de consommation, des baisses de production d'œufs commercialisables et une diminution d'éclosabilité des œufs (Gautier-Bouchardon et Kempf, 2008). De plus, non contrôlées, ces infections prédisposent les sujets aux infections secondaires entraînant de fortes mortalités (Kempf, 1997).

Les deux principaux mycoplasmes responsables d'affections chez la poule sont *Mycoplasma gallisepticum* (MG) et *Mycoplasma*

*synoviae* (MS) (Kempf, 1997). *Mycoplasma gallisepticum* (MG) est responsable de la maladie respiratoire chronique de la poule. Son pouvoir pathogène est le plus souvent exacerbé lors d'association avec d'autres agents infectieux tels que les virus (de la maladie de Newcastle, de la bronchite infectieuse, de la rhinotrachéite infectieuse), les bactéries (*Echerichia coli*, *Haemophilus*, *Pasteurella*) ou les champignons (*Aspergillus*). Dans les conditions naturelles, l'infection par MG peut rester subclinique ou se limiter à une simple séroconversion. Dans d'autres cas, elle provoque des symptômes respiratoires qui comprennent principalement des éternuements, du jetage nasal et oculaire, et de la dyspnée. Chez les poules pondeuses, le taux de ponte peut être fortement diminué. On constate aussi une faible éclosabilité et des mortalités à l'éclosion allant de 5 à 10 %. Chez les poulets de chair, la croissance est fortement ralentie. Les lésions observées peuvent se limiter à la présence d'une quantité importante de mucus ou une inflammation catarrhale des premières voies respiratoires et un œdème des sacs aériens. Aussi, une inflammation fibrineuse des sacs aériens et des différents organes internes (péritoine, capsule hépatique) peut être observée.

*Mycoplasma synoviae* (MS) est responsable d'infections articulaires chez les poules. Comme pour MG, le pouvoir pathogène de MS est exacerbé lors d'associations avec des virus et des bactéries. Lors d'atteintes articulaires aiguës, les oiseaux paraissent faibles et pressentent une pâleur de la crête et des barbillons ainsi que des articulations volumineuses, notamment au niveau des pattes, parfois des ailes. Dans les formes articulaires chroniques, les articulations restent tuméfiées et les oiseaux répugnent à se déplacer.

Le système moderne d'aviculture étant une activité de profit, il importe que toutes contraintes de production soient levées. Les maladies réputées meurtrières comme la maladie de Newcastle, la maladie de Gumboro et les salmonelloses étant déjà prises en charge à travers des plans de prophylaxie de routine adaptés, il importe maintenant de s'attaquer aux maladies ayant un impact sur la productivité. C'est ce qui justifie notre intérêt pour les mycoplasmoses aviaires.

Cette étude avait pour objectif principal de déterminer la prévalence des mycoplasmoses aviaires dans les élevages avicoles améliorés et de confirmer leur importance économique en zones périurbaines du Mali.

## Matériels et Méthodes

### Zones de l'étude

L'étude a été menée dans les élevages avicoles améliorés des zones périurbaines de Ségou, Sikasso et du District de Bamako au Mali (tableau 1). Le choix de ces sites a été opéré en tenant compte de l'importance des effectifs. Les exploitations avicoles ont été retenues par choix raisonné en fonction de l'adhésion et la coopération des aviculteurs au projet avec l'appui des structures techniques de l'élevage. Les fermes retenues faisaient l'objet de suivi vétérinaire particulier, avec l'application des plans de prophylaxies de routine visant à contrôler les pathologies majeures portant sur le déparasitage (coccidioses), l'antibiothérapie (salmonelloses, colibacillooses) et la vaccination (maladie de Newcastle, maladie de Gumboro, bronchite infectieuse et variole aviaire). Les poules bénéficiaient d'aliment équilibré et étaient de souche Leghorn, fournies sous forme de poussins d'un jour, par «Mali Poussins» une compagnie locale productrice de poussins. La taille des bandes prélevées variait de 1000 à 3000. Au moment de l'enquête, elles étaient en fin de carrière.

### Récolte des sérums

Au total, 360 sérums ont été prélevés sur des poules pondeuses en fin de carrière. Les prélèvements ont été effectués de façon aléatoire ou selon la bonne volonté des propriétaires. Le sang a été prélevé dans des tubes vacutainer stériles, puis mis à coaguler et centrifuger sur place. Après centrifugation, les sérums ont été extraits, aliquotés et transportés sous glace au LCV où ils ont été conservés à  $-20^{\circ}\text{C}$  jusqu'au moment du test.

### Test sérologique

Les échantillons ont été testés à l'aide de kits ELISA indirect (ProFLOK<sup>®</sup> MG et ProFLOK<sup>®</sup> MS), spécifiques pour la détection

des anticorps contre MG et MS dans le sérum des poules, et fourni par Synbiotics Corporation. Les kits sont livrés avec microplaques déjà sensibilisées avec l'antigène bactérien inactivé de MG ou MS. Le kit fourni contient également les tampons de dilution et de lavage, le conjugué anti-poule-IgG (H+L) marqué à la peroxydase de raifort, les sérums de contrôle positif et négatif à MG ou MS, le substrat chromogène et la solution d'arrêt. Le test a été fait selon le protocole du kit. Brièvement, les sérums à tester et les sérums de contrôle ont été prédilué à 1:50 puis transférés dans la plaque de test. Après incubation, lavages et révélation, les densités optiques (DO) des puits, qui sont directement proportionnelles à la quantité totale d'anticorps spécifiques contre MG ou MS présents dans les échantillons testés, ont été déterminées à l'aide d'un lecteur Multiskan avec un filtre de 405 nm. La moyenne des DO des puits du contrôle positif a été ajustée en y soustrayant la moyenne des DO des puits du contrôle négatif.

La valeur diagnostique de chaque échantillon (VD) a été déterminée selon la formule suivante:

$$VD = \frac{\text{DO sérum échantillon} - \text{Moyenne DO du control négatif}}{\text{Moyenne DO corrigée du control positif}}$$

L'interprétation des résultats a été donnée comme suit:

VD < 0,199 → négatif; VD = 0,200 à 0,599 → douteux; VD > 0,600 → positif

### Analyse statistique

Les données ont été traitées à l'aide du logiciel R version 2.9.2. L'existence de différence significative a été déterminée à l'aide de la méthode linéaire générale.

**Tableau 1 :** Prévalence des anticorps anti- *Mycoplasma synoviae* (MS) et anti- *Mycoplasma gallisepticum* (MG) par localités

Localités	Nombre de sérums testés	Nombre de sérums positifs (%)	
		MS	MG
District de Bamako	123	108 (87,80)	51 (41,46)
Ségou	63	45 (71,42)	5 (7,93)
Sikasso	174	90 (51,72)	57 (32,75)
<b>Total</b>	<b>360</b>	<b>243 (67,50)</b>	<b>113 (31,38)</b>

Résultats et Discussion

Les résultats obtenus au cours de la présente enquête conduite pour la première fois pour évaluer la prévalence des mycoplasmoses aviaires dans les élevages avicoles améliorés en zones périurbaines du Mali ont montré un taux global de prévalence de 67,50 % pour MS et de 31,39 % pour MG (tableau 1). Ces prévalences étaient proches de celles obtenues lors d’une enquête similaire réalisée au Sénégal qui indiquait 20 à 28 % pour MS et 4 à 5 % pour MG chez les poules pondeuses (en aviculture industrielle selon les saisons); 50 à 60 % pour MS et 43 à 49 % pour MG (en aviculture rurale selon les saisons) (Arbelot *et al.*, 1997). Ces résultats peuvent aussi être rapprochés de ceux obtenus en aviculture rurale au Burkina (40 % pour MS et 18 % pour MG) (Berthé, 1997) et au Soudan (42 % pour MS et 43 % pour MG) (El Hassan *et al.*, 1989).

D’une manière générale, il existe peu de données sur les pathologies aviaires au Mali et à la connaissance des auteurs aucune donnée de prévalence n’est actuellement disponible pour les mycoplasmoses aviaires. Les enquêtes sérologiques menées par Tounkara *et al.*, (1995) sur les principales viroses au Mali ont montré une prévalence élevée de la maladie de Newcastle et de la variole. Plus récemment, Sylla *et al.*, (2003) ont étudié les causes de mortalité et de morbidité de la volaille en milieu rural au Mali et leurs résultats indiquent que la maladie de Newcastle, la variole, la maladie de Gumboro, les colibacilloses, les salmonelloses et les parasites internes sont les principales pathologies identifiées. Les résultats de diagnostic effectué pendant ces dernières années au LCV de Bamako (LCV, 2009) sur des prélèvements provenant des élevages avicoles

en zone périurbaine du District de Bamako ont, pour la plupart des cas, été positifs pour la maladie de Newcastle, la maladie de Gumboro, le choléra, les colibacilloses, les salmonelloses et les parasitoses internes. Ces résultats, bien que confirmant ceux des 2 études antérieures, sont biaisés et ne font pas ressortir entièrement la carte pathologique de ces élevages. En effet, dû à l’insuffisance des moyens d’investigation des cliniciens, les échantillons reçus au laboratoire sont analysés, dans la plupart des cas, pour suspicion des pathologies à fort taux de mortalité. D’autres maladies plus discrètes dans leur évolution, comme les mycoplasmoses, sont rarement prises en compte par les éleveurs et les cliniciens.

Pour l’ensemble des sérums testés, la prévalence sérologique globale pour MS (67,50 %) a été significativement plus élevée que celle pour MG (31,38 %) avec une p-value inférieure à 0,01 ( $p < 2,2 \times 10^{-16}$ , test exact de Fisher). Différents facteurs pourraient expliquer cette différence de fréquence. Il a été démontré que MS persiste plus longtemps dans l’environnement que MG (Marois, 2001) et que la diffusion de MS est généralement plus rapide que celle de MG (Kempf, 1997). Aussi, il a été démontré que l’infection par MS peut en général atteindre une grande partie des oiseaux de l’élevage (90-100 %), mais avec un faible taux de mortalité des sujets ( $< 1\%$ ) (Kempf, 2006). Compte tenu du mode de contamination de ces maladies qui peut être par contact direct entre sujets par voie respiratoire et/ou conjonctivale ou par contact indirect par l’homme et le matériel d’élevage, une forte fréquentation des exploitations par les revendeurs des œufs (cyclistes) peut être impliquée dans sa dissémination entre les exploitations. Cette tendance où MS est plus

fréquente que MG avait été trouvée au Sénégal (23 % pour MS et 2 % pour MG) (Arbelot et al., 1997) et au Burkina Faso (40 % pour MS et 18 % pour MG) (Berthé, 1997). Par contre, les études menées par Boussetta et al., (1997) en Tunisie, indiquaient le contraire (19 % pour MS et 36,5 % pour MG). Une technique d'analyse différente que celle utilisée dans la présente enquête pourrait expliquer cette différence.

L'étude a également montré une forte variation de la prévalence sérologique pour toutes les 2 espèces de mycoplasmes entre les localités enquêtées. Pour MS, la plus forte prévalence sérologique a été enregistrée à Bamako avec un taux de 87,80 % contre un taux de 51,72 % à Sikasso. Cette différence a été statistiquement significative ( $p < 3,959 \times 10^{-10}$ ). La prévalence sérologique due à MG, malgré qu'elle soit faible au niveau des localités comparée à celle due à MS, a également significativement varié de 7,93 % (Ségou) à 41,46 % (Bamako) ( $p < 1,639 \times 10^{-5}$ ). Ceci démontre que, bien que tous les sites soient plus exposés à MS qu'à MG, le risque est plus élevé à Bamako pour ces mycoplasmoses que dans les autres localités de l'étude. La forte concentration des fermes avicoles dans le District de Bamako compte tenu de sa proximité de grands marchés de consommation pourrait favoriser la diffusion des mycoplasmes et expliquer cette différence.

## Conclusion

La présente enquête sur la prévalence sérologique des mycoplasmoses aviaires chez les poules pondeuses dans les fermes avicoles améliorées en zone périurbaine du Mali a permis d'établir une prévalence sérologique globale de 67,50 % pour MS et de 31,39 % pour MG. La prévalence de l'infection semble relativement plus élevée dans le District de Bamako que dans les régions de Ségou et Sikasso. Cette étude est une première au Mali et indique, à partir d'une enquête sérologique basée sur la détection des anticorps, l'existence de mycoplasmoses aviaires au Mali. Puisque la vaccination contre les mycoplasmoses aviaires n'est pas pratiquée dans ce pays, ces résultats reflètent certainement l'infection et méritent d'être ultérieurement consolidés en menant des recherches dans le sens de l'isolement. Une

étude épidémiologique plus précise devrait pouvoir renseigner sur l'importance nationale des mycoplasmoses dans toutes les régions du Mali.

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## **GASTRO-INTESTINAL PARASITES OF WARTHOGS (*PHACOCHOERUS AFRICANUS*) FROM THE NAZINGA GAME RANCH OF BURKINA FASO**

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### **Summary**

A survey was conducted to assess the prevalence of gastrointestinal parasites in warthogs from the Nazinga Game Ranch of Burkina Faso. The study revealed that Eight different nematodes and one cestode species were present in the gastrointestinal tracks of the animals; . In the stomach, *Simondsia paradoxa* was found at prevalence as high as 83.3% while both *Hyostrongylus rubidus* and *Gnathostoma* sp. were each present in 5.6% of the cases. The small intestine was infested by *Globocephalus* sp. (55.6%), *Stilesia globipunctata* (22%), and *Ascaris phacochoeri* (5.6%). The large intestine harboured *Probstmayria vivipara* at a maximum prevalence of 100%, followed by *Murshidia* sp. (88.9%), and *Oesophagostomum* sp. (72%). All the 18 warthogs studied were infested by several types of parasites. Number of worms per animal showed important burden for *Murshidia* sp. ( $4817 \pm 2348$ ) and *S. paradoxa* ( $995 \pm 1979$ ), also for *Oesophagostomum* sp. ( $141 \pm 137$ ), and particularly for *P. vivipara* ( $826074 \pm 1232650$ ). Despite the presence of worms, all warthogs studied were in good physical status with an average blood-letting weight of  $60.7 \pm 15$  kg and a calculated killing out percentage of 43.3%, which seems to indicate a good potential for meat production.

## **PARASITES GASTRO-INTESTINAUX DES PHACOCHÈRES (*PHACOCHOERUS AFRICANUS*) DU RANCH DE GIBIER DE NAZINGA AU BURKINA FASO**

### **Résumé**

Une étude des parasites gastro-intestinaux, menée sur des phacochères du Ranch de gibier de Nazinga au Burkina Faso, a permis l'identification de huit espèces de nématode et d'une espèce de cestode, ainsi qu'une estimation de leur prévalence et importance numérique. Dans l'estomac, l'étude a identifié une forte prévalence de *Simondsia paradoxa* (83,3%), ainsi que la présence de *Hyostrongylus rubidus* et *Gnathostoma* sp avec une prévalence de 5,6% chacun. L'intestin grêle était infesté par *Globocephalus* sp (55,6%), *Stilesia globipunctata* (22%), et *Ascaris phacochoeri* (5,6%). Le gros intestin hébergeait *Probstmayria vivipara* à une prévalence maximale de 100%, suivi de *Murshidia* sp. (88,9%) et *Oesophagostomum* sp. (72%). Chacun des 18 phacochères étudiés était infesté par plusieurs types de parasites. La charge parasitaire était importante pour *Murshidia* sp. ( $4817 \pm 2348$  nombre moyen par animal), *S. paradoxa* ( $995 \pm 1979$ ), *Oesophagostomum* sp. ( $141 \pm 137$ ), et plus particulièrement *P. vivipara* ( $826074 \pm 1232650$ ), un parasite vivipare. Malgré la présence de vers, la quasi-totalité des phacochères étudiés étaient en bon état physique, avec un poids saigné moyen de  $60,7 \pm 15$  kg et un pourcentage de viande calculé de 43,3%, ce qui semble indiquer un bon potentiel de production de viande.

## Introduction

Wildlife constitutes an important socio economic asset in west Africa as it contributes to food security, culture, tourism, science and medicine. In Burkina Faso, almost all the wild game species of the Soudanian area can be found. According to Chardonnet *et al.* (1995), there are about 147 mammal species, 497 bird species, and numerous reptile species. The important task of management and organisation of wild game ranches undertaken by the appropriate authorities of the country can be compromised if problems related to the pathology of the animals are not addressed. Infestation of animals by helminths, protozoa, and arthropods can lead to an important economic loss and a negative impact on human health (Cox and Todd, 1962).

In order to design better and successful strategies to control these parasites it is important to assess their typology and prevalence; this study was therefore designed to assess the prevalence of gastro intestinal parasites in warthogs (*Phacochoerus africanus*) in the Nazinga Wild Game Ranch of Burkina Faso.

## Materials and methods

### *Study site and animals*

The study was conducted on the Nazinga Game Ranch of Burkina Faso, which covers 940 km<sup>2</sup> and is located at 200 km away from Ouagadougou, the capital of the country. Its location is between 11°01' to 11°11' latitude north and between 01° 18' to 01°48' longitude west (Mattioli *et al.*, 1998). The ranch has 3 rivers which flow only during the rainy season. The climate is in between the soudano-sahelian and the soudano-guinean climates with an average rainfall of about 965 mm, from May to October. Maximum and minimum temperatures are 34° and 22°C respectively (Guinko, 1984).

The eighteen (18) warthogs studied were collected during 6 hunting periods from 2000 to 2005 (December to May). Among those, 4 were young with an age range of 2 to 3 years, with blood-letting weights ranging from 34 to 51 kg. The other 14 were adult

with an age range of 4 to 8 years and a blood-letting weights ranging from 48 to 91 kg. They all harboured many ticks on their bodies, were all in good physical status with the exception of one which was sick and dying. The mean blood-letting weight of the animals examined was 60.7±15 kg and the calculated killing out percentage was 43.3%.

### *Parasitological examination*

The parasites identified and counted were collected from aliquots taken from the contents of different parts of the digestive tube: the stomach, the small and large intestines. During the sampling process, segments of cestodes seen were systematically added to the aliquots for the final evaluation of parasite's prevalences.

Samples were collected from digestive tracks of death warthogs at the head-quarter of the ranch approximately 6 to 12 hours after the gun shot. The whole stomach as well as the small and the large intestines were isolated by double ligatures using strong cotton string, and carefully trimmed from fat and mesenteries, following slight modifications of the method already described by Belem *et al.* (2005). Each of the three parts was placed in a clean plastic bag for further processing. Each part of the digestive tract was cut opened in graduated buckets and under a running tap water. Contents and washing were brought to a volume of 10 l for the stomach and 5 l for the small and large intestines. Then, 10% aliquots were taken in clean bottles and labelled. Formaldehyde was added in each bottle to approximate 10% (v/v).

All formalin-fixed samples were stored at room temperature until use. Before use, the aliquot was washed through a sieve with a 38 µm mesh. Using scribed glass Petri dishes, a stereoscopic dissecting microscope, and needles, parasites were recovered, then transferred to labelled microscope slides with drops of lactophenol until identification and enumeration with the aid of compound microscope (Belem *et al.*, 2005). During the process of worm recovery, when too many parasites were noticed in a sample, a second aliquot of the first aliquot was made and used. Identification of parasites was performed according to several keys and descriptions

of which the most important were Euzeby (1961, 1963, 1966), Anonymous (1974), Soulsby (1982), Reinecke (1983), Graber and Perrotin (1983), and Georgi and Georgi (1990).

#### Statistical analysis

Data were all collected using the Microsoft Excel software. Mean worm numbers and their standard deviations as well as other statistics were performed using the Statistical Analysis System software (SAS Institute, 1987). Analyses of variance were performed using the ANOVA test. Means were separated according to the Student-Newman-Keul (SNK) mean separation test at a probability level of 5%. The Ki-square ( $\chi^2$ ) test was used to compare prevalences.

## Results

#### Identified gastro-intestinal parasites

Table 1 presents the identified parasite species with their anatomical locations and global prevalence for the period of study. Nine different parasite species were found, one cestode (*Stilesia globipunctata*) and eight nematodes (*Simonsia paradoxa*, *Gnathostoma* sp., *Hyostromylus rubidus*, *Probstmayria vivipara*, *Globocephalus* sp., *Ascaris phacochoeri*, *Murshidia* sp., and *Oesophagostomum* sp.). Only adult males and females were found for most of the parasite species, but for *S. paradoxa*, *Globocephalus* sp., and *Murshidia* sp. four larval stage parasites (L4) were also found. Pre-adult stages (L5) were found for *S. paradoxa*.

#### Monthly prevalences and mean worm numbers

##### Parasites of the stomach

In the stomach of examined warthogs, *S. paradoxa* was the most prevalent species (83.3%). *H. rubidus* and *Gnathostoma* sp. had a lower prevalence (5.6%). The month of January showed the highest prevalences for all worm species as compared to February and March. *S. paradoxa* was still the most numerous with a mean number of  $995 \pm 1979$ . *H. rubidus* and *Gnathostoma* sp. were not found in great number in the stomach; their mean numbers showed respectively 80 and 120 per animal (Table 2). Prevalences and mean worm burdens

were not significantly different mostly because of large standard deviations.

##### Parasites of the small and large intestines

In the small intestine (Table 3), *Globocephalus* sp. had the highest prevalence (55.6%) while *S. globipunctata*, the only cestode species found, and *A. phacochoeri* showed respective prevalences of 22 and 5.6%. *Globocephalus* sp. was most prevalent in January (80%) and both *S. globipunctata* and *A. phacochoeri* were most prevalent in March with respectively 42.9% and 14.3%. For worm burdens, *A. phacochoeri* and *Globocephalus* sp. were found at respective mean numbers of 80 and  $72 \pm 37$  per animal. *Globocephalus* sp. had the highest mean number in February ( $93.33 \pm 24$ ), and *A. phacochoeri* was only found in March at 80 worms per animal.

For the warthogs' large intestines (Table 3), a particular mention has to be made for *P. vivipara* which was the most prevalent species (100%) and found in all animals. *Murshidia* sp. had a prevalence of 88.9% and *Oesophagostomum* sp., 72%. Furthermore, *P. vivipara* was found in the large intestines at a huge mean number of  $826074 \pm 1232650$  per animal with one warthog found with 3896720 parasites in January. *Murshidia* sp. and *Oesophagostomum* sp. were both most prevalent in March with respectively the prevalences of 100% and 85.7%. *Murshidia* sp. was found at a mean number of  $4817 \pm 2348$  and *Oesophagostomum* sp. at the lowest mean number per animal of  $141 \pm 137$ . *Oesophagostomum* sp., the least numerous nematode in the large intestine showed a steady decrease in mean numbers from January to March and *Murshidia* sp. had its highest mean number in February ( $6870 \pm 2912$ ).

For the parasites of the large intestines also, prevalences and mean worm burdens were not significantly different mostly because of large standard deviations.

## Discussion

In the present survey, 9 different helminth species (8 nematodes and 1 cestode) were found in the gastro-intestinal tract of warthogs hunted from the Nazinga Game Ranch of Burkina Faso. The 8 nematode species

**Table 1:** Parasite species identified from warthogs and their global prevalences

Part of the digestive tract	Identified parasite species	Global prevalences (in %)
Stomach	Simondsia paradoxa	83.3
	Hyostromgylus rubidus	5.6
	Gnathostoma sp.	5.6
Small intestine	Globocephalus sp.	55.6
	Ascaris phacochoeri	5.6
	Stilesia globipunctata	22
Large intestine	Probstmayria vivipara	100
	Murshidia sp.	88.9
	Oesophagostomum sp.	72

**Table 2:** Prevalences and mean worm numbers of parasites in the stomach

	Simondsia paradoxa		Hyostromgylus rubidus		Gnathostoma sp.	
	Numbers	Prevalence (%)	Numbers	Prevalence (%)	Numbers	Prevalence (%)
January	512±493 (40-1200)	100	80 (80-80)	20	120 (120-120)	20
February	520±605 (40-1200)	60	-	-	-	-
March	1760±3072 (160-8000)	85.7	-	-	-	-
Total	995±1979 (40-8000)	83.3	80 (80-80)	5.6	120 (120-120)	5.6

were: *S. paradoxa*, *Gnathostoma sp.*, *H. rubidus*, *P. vivipara*, *Globocephalus sp.*, *A. phacochoeri*, *Murshidia sp.*, and *Oesophagostomum sp.* The only cestode was *S. globipunctata* in the small intestine. With the exception of *Globocephalus sp.*, the same types of parasites have been recovered from warthogs by Horak *et al.* (1983) in South West Africa/Namibia and Troncy *et al.* (1972) in Central Africa. However Troncy *et al.* (1972) have also found *Globocephalus sp.* in a different wild pig species, the *Potamochoerus porcus*. Overall, the parasites on warthogs were similar to those described on domestic pigs by Soulsby (1982).

*S. paradoxa* described for the first time in tropical Africa, particularly in Mali, by Bussieras (1973) was found in the stomach at a high prevalence of 83.3% and also in great numbers. *H. rubidus* and *Gnathostoma sp.* did not seem to be very important worm species for the warthogs of the Nazinga Ranch. Also, at this location, warthogs did not seem to be very much infested by adult cestodes because

only one species was found, *S. globipunctata*, at a prevalence of 22%. *Globocephalus sp.* was not numerous in the small intestines but was found on about half of the animals at necropsy (55.6%).

*P. vivipara*, *Murshidia sp.*, and *Oesophagostomum sp.*, parasites of the large intestines, were found at high prevalences, respectively 100, 88.9, and 72%. In South West Africa/Namibia, Horak *et al.* (1983) have also found *P. vivipara* at a prevalence of 100%, and *Oesophagostomum roubaudi* at 84.2%. At a different location, in the Eastern Transvaal, Boomker *et al.* (1991) have recovered *P. vivipara*, *Oesophagostomum mwanzae*, and *Murshidia hamata* at the highest prevalences of 100% for each. The particular biology of the parasite *P. vivipara* which is viviparous and can undergo several cycles of auto-infestation on the same animal, explains easily the presence of enormous numbers and the high prevalences of 100% in the large intestines of warthogs.

The study showed that the most

**Table 3:** Prevalences and mean worm numbers of parasites in the small and large intestines

	Small Intestine					
	<i>Globocephalus</i> sp.		<i>Ascaris phacochoeri</i>		<i>Stilesia globipunctata</i>	
	Numbers	<sup>a</sup> Prev (%)	Numbers	Prev (%)	Prev (%)	
January	60±40 (40-120)	80	-	-	20	
February	93.33±24 (80-120)	60	-	-	-	
March	66,66±46 (40-120)	42,9	80 (80-80)	14,3	42,9	
<b>Total</b>	<b>72±37 (40-120)</b>	<b>55.6</b>	<b>80 (80-80)</b>	<b>5.6</b>	<b>22</b>	

	Large Intestine					
	<i>Probstmayria vivipara</i>		<i>Murshidia</i> sp.		<i>Oesophagostomum</i> sp.	
	Numbers	Prev (%)	Numbers	Prev (%)	Numbers	Prev (%)
January	1696200±1425228 (10520-3896720)	100	5030±1583 (3440-7120)	80	200±189 (40-440)	80
February	101752±224316 (40-503000)	100	6870±2912 (3680-9360)	80	160±174 (40-360)	60
March	643820±1275994 (120-2557800)	100	3549±1888 (1360-6920)	100	93±86 (40-240)	85,7
<b>Total</b>	<b>826074±1232650 (40-3896720)</b>	<b>100</b>	<b>4817±2348 (1360-9360)</b>	<b>88.9</b>	<b>141±137 (40-440)</b>	<b>72</b>

<sup>a</sup>Prev = Prevalence in %

numerous gastro-intestinal worms in the warthogs are *P. vivipara*, *Murshidia* sp., *S. paradoxa*, and *Oesophagostomum* sp. Like in the Nazinga Game Ranch, Horak *et al.* (1983) have also found millions of *P. vivipara* in the large intestines of warthogs in South West Africa/Namibia. In January, the coolest month during the period of study, an animal was found with more than 3millions *P. vivipara* worms. It is also interesting to notice that none of the animal was free of any type of parasite, and that poly-infestations were the rule. Furthermore, all the animals studied harboured ticks on their bodies. Nonetheless, almost all the warthogs collected were physically in good status with an average blood-letting weight of 60.7±15 kg and a calculated killing out percentage of 43.3%, which seems to indicate a good potential for meat production like already stated by Mattioli *et al.* (1998).

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# THE EFFECT OF WALNUT (*TETRACARPIDIUM CONOPHORUM*) LEAF AND ONION (*ALLIUM CEPA*) BULB RESIDUES ON THE TISSUE BACTERIOLOGICAL CHANGES OF *CLARIAS GARIEPINUS* JUVENILES

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**Running title:** Bacteriological changes of *Clarias gariepinus*

## Abstract

In this study, the effect of walnut leaf (WL) and onion bulb (OB) residues on tissue bacteriology of *Clarias gariepinus* juveniles by dietary intake was investigated. Nine experimental diets: control (0%), OB2 (0.5%), OB3 (1.0%), OB4 (1.5%), OB5 (2.0%), WL6 (0.5%), WL7 (1.0%), WL8 (1.5%) and WL9 (2.0%) were formulated and replicated thrice at 40% crude protein. Fish (mean weight  $7.4 \pm 0.02$ g) were fed twice daily at 3% body weight for 12 weeks. Microbiological analyses of water and fish (skin, gill, intestine and liver) and organ index (liver, spleen, kidney and heart) were investigated. Data were analysed using descriptive statistics and ANOVA at  $p=0.05$ . Results of enterobacteriaceae and total viable count from this study revealed that bacterial loads on the water and fish of the experimental tanks were more affected by *A. cepa* and *T. conophorum* than the control for 4, 8 and 12 weeks and were significantly different ( $P<0.05$ ) from the control. The values decreased in treated groups as the levels of inclusion (0.5%, 1.0%, 1.5% and 2.0%) increased and as the months increased. Also, organ index showed that the liver, heart, kidney and spleen were not significantly increased in all the treated groups and the control. The results suggest that walnut leaf and onion bulb residues inclusion in the diet of *Clarias gariepinus* could be a potential, less expensive and promising dietary supplementation that would positively influence growth, reduce and prevent bacterial infections in fish culture.

**Keywords:** microbial load, walnut leaf, onion bulb, *Clarias gariepinus*, bacteria

## L'EFFET D'UN APPORT ALIMENTAIRE DE RESIDUS DE FEUILLES DE NOYER (*TETRACARPIDIUM CONOPHORUM*) ET DE BULBES D'ONION (*ALLIUM CEPA*) SUR LA BACTERIOLOGIE DES TISSUS DE *CLARIAS GARIEPINUS* JUVENILES

**Titre courant :** Changements bactériologiques de *Clarias gariepinus*

## Résumé

La présente étude a examiné l'effet d'un apport alimentaire de résidus de feuilles de noyer (WL : walnut leaf) et de bulbes d'oignon (OB : onion Bulb) sur la bactériologie des tissus de *Clarias gariepinus* juvéniles. Neuf régimes expérimentaux - témoin (0%), OB2 (0,5%), OB3 (1,0%), OB4 (1,5%), OB5 (2,0%), WL6 (0,5%), WL7 (1,0%), WL8 (1,5%) et WL9 (2,0%) - ont été préparés et répétés trois fois avec une teneur en protéines brutes de 40%. Des poissons (poids moyen  $7,4 \pm 0,02$  g) ont été nourris à 3% du poids corporel deux fois par jour pendant 12 semaines. Des analyses microbiologiques de l'eau et des poissons (peau, branchies, intestin et foie) ont été effectuées, et l'indice d'organes (foie, rate, reins et cœur) a été étudié. Les données ont été analysées à l'aide de statistiques descriptives et de l'ANOVA à  $p = 0,05$ . Les résultats du dénombrement des enterobactériacées et le total des comptages viables de cette étude ont révélé que les charges bactériennes sur l'eau et les poissons des bassins expérimentaux ont été plus affectées par *A. cepa* et *T. conophorum* par rapport au groupe témoin pendant 4, 8 et 12 semaines et étaient significativement différentes ( $P < 0,05$ ) de celles du groupe témoin. Les valeurs ont diminué dans les groupes traités au fur et à mesure de l'augmentation des niveaux d'inclusion (0,5%, 1,0%, 1,5% et 2,0%) et des mois. De plus, l'indice d'organes a montré que le foie, le cœur, les reins et la rate n'avaient pas significativement

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augmenté de volume, que ce soit dans tous les groupes traités ou le groupe témoin. Ces résultats portent à croire que l'inclusion de résidus de feuilles de noyer et de bulbes d'oignons dans le régime alimentaire de *Clarias gariepinus* pourrait être envisagée comme une supplémentation alimentaire potentielle, moins coûteuse et prometteuse, capable d'avoir une influence positive sur la croissance, de réduire et de prévenir les infections bactériennes dans l'élevage de poissons.

**Mots-clés:** charge microbienne ; feuille de noyer ; bulbe d'oignon ; *Clarias gariepinus* ; bactéries

## Introduction

The main goals of aquaculture industry are to optimize growth and to produce high-quality fish. The outbreak of diseases in fish farming is a major obstacle worldwide and this brought economic loss to the industry. The high susceptibility of fish to stress and the rapid spread of diseases in water have forced fish farmers to concentrate their efforts on maintaining fish against infectious disease in order to achieve sustainable economic performances. The epithelial surfaces of fish, such as those of skin, gills or gastrointestinal tract are the first contact areas for potential pathogens (Iijima *et al.*, 2003, Narvaez *et al.*, 2010). Prophylaxis and treatment using antibiotics in aquaculture have negative impacts, one of which is the emergence of bacterial resistance. Considering the potential threat of diseases to human and animal health, issues associated with the use of antibiotics, disease management should therefore concentrate on environmental-friendly, preventative methods such as the use of immunostimulants.

Using immunostimulants can enhance activities in the non-specific defense mechanism (Anderson, 1992), increase resistance to infectious diseases by enhancing innate humoral and cellular defense mechanisms and indirectly to cause growth improvement in fish (Galindo-Villegas and Hosokawa, 2004). Presently, attention is given to immunostimulants and many different immunostimulants have been found to be effective in various fish species (Gatta *et al.*, 2001, Li *et al.*, 2004, Rairakhwada *et al.*, 2007, Cerezuela *et al.*, 2009). Organic fish culturing has become popular over the last decade and therefore natural immunostimulants have received even more attention. Some researchers observed positive results in the improvement of immune system in fish fed with natural immunostimulants (Dugenci *et al.*, 2003, Divyagnaneswari *et al.*, 2007, Yin *et al.*, 2009)

Walnut leaf and onion bulb as plant immunostimulants could be considered as immunostimulants in cultured fish as they possess high antimicrobial and antibacterial effects (Ajaiyeoba and Fadare 2006, Azu and Onyeagba, 2007). This study was carried out to evaluate the possible effect of walnut leaf and onion bulb residues as potential antimicrobials in the farming of *Clarias gariepinus*

## Materials and Methods

### Plant Collection and Identification

Onion bulbs were purchased from Bodija market in Ibadan, Nigeria. Walnut leaf was obtained from a farm at Oka -Akoko, Nigeria. They were authenticated at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, where a voucher specimen was deposited under FHI 107515.

### Preparation and Extraction of Plant Materials

#### Onion extraction

The onion bulbs were washed with distilled water and allowed to air dry at ambient temperature (25°C) for one hour. The dry outer coverings of the onions were manually peeled off, washed and extracted as described by Azu and Onyeagba, (2007). 200g of the fresh onion bulbs were blended into fine powder and soaked in 100ml of 95% ethanol for 24hrs. The pulp obtained was left in a clean, sterile glass container, shaken vigorously to allow for proper extraction, filtered using a sterile muslin cloth after which the residue was obtained, air-dried and stored at 4°C until required.

#### Walnut leaf extraction

The extraction was carried out as described by Ajaiyeoba and Fadare (2006). The air – dried walnut leaves were ground with a hammer mill to fine powder. 200g of the

powder was soaked in 100ml of 80% methanol for 72 hours, properly mixed with methanol, filtered using a sterile muslin cloth after which the extract was obtained. The residue was air – dried and stored at 25°C until required.

### Media Preparation

All media used were prepared according to manufacturer's instruction as follows:

- MacConkey agar: This agar was prepared by suspending 52g in 1 litre of distilled water. It was brought to boil to dissolve completely then sterilized by autoclaving at 121°C for 15 minutes.
- Nutrient agar: This agar was prepared by suspending 28g in 1 litre of distilled water and then sterilized by autoclaving at 121°C for 15 minutes.
- Mueller Hinton agar: This agar was prepared by suspending 36g in 1 litre of distilled water and then sterilized by autoclaving at 121°C for 15 minutes.
- Nutrient broth: This broth was prepared by suspending 25g in 1 litre of distilled water and then sterilized by autoclaving at 121°C for 15 minutes.
- Peptone water: This was prepared by suspending 15g in 1 litre of distilled water and then sterilized by autoclaving at 121°C for 15 minutes.

All these media were allowed to cool after sterilization to about 45°C before pouring into Petri dishes.

### Preparation of Experimental Diets

The mean proximate composition of the experimental diet was 40.0% crude protein, 15.9% ether extract, 15.7% ash, 7.4% moisture, and 20.9% NFE. Nine experimental diets were prepared by incorporating walnut leaf and onion bulb residues at the following inclusion levels; 0 (control), 0.5%, 1.0%, 1.5% and 2.0% respectively. Feed ingredients such as fishmeal, soybean, maize, starch, vegetable oil, Di calcium phosphate (DCP), salt and vitamin- mineral premix were added and the dry ingredients mixed thoroughly in a mixer. Water was added and the resulting dough pelleted. The pellets were sun –dried, and stored in airtight

containers at room temperature to prevent mould formation until required.

### Microbiological analysis

Water samples from the aquaria were collected monthly in sterile glass bottles. Peptone water 0.1% was used for serial dilution. 1ml of water sample was added to 9ml sterile peptone water to 10<sup>-1</sup> and then diluted to 10<sup>-4</sup>. Each diluent (1ml) was poured in two Petri dishes; one received plate count agar for total bacterial count using the pure plate count method according to the standard methods for the examination of water and wastewater (APHA, 1985), the second Petri dish received MacConky agar for total coliforms count according to Hitchins *et al.*, (1995). Petri dishes were gently tapped on the sides for a few times. Petri dish for total coliform count and that of the dishes of total bacterial count were incubated at 37°C for 24h.

Fish samples (skin, liver, gill and intestine) were collected monthly during the experimental period for bacteriological examination with through asepsis. In accordance with the American Public Health Association, 1g of fish sample was grained in 9ml sterile peptone water in the mortar. 1ml of the suspension was diluted by peptone water to 10<sup>-4</sup>. Each diluent (1ml) was poured in two Petri dishes; one received plate count agar and the other received MacConky agar. The incubation period was 24h at 37°C. After incubation of water and fish sample dishes the colonies were counted using colony counter. Total viable count and enterobacteriaceae were determined, the result were expressed in log<sub>10</sub> CFU/ml and log<sub>10</sub> CFU/g for water and fish, respectively.

### Organ Index

Three fish from each experimental treatment were killed by rapid cervical chopping and weighed. The liver, kidney, intestine and spleen were removed and weighed and the average was calculated. Moreover, the hepatosomatic and splenosomatic indices were calculated according to Fox *et al.*, (1997)

Organ-somatic index = [organ weight (g)/body weight (g)] × 100.

**Table 1:** Enterobacteriaceae and total viable counts (log 10CFU/ml) of water samples treated with onion bulb and walnut leaf

Treatment	4 Weeks		8 Weeks		12 Weeks	
	Enterobacte- riacea	Total viable counts	Enterobacte- riacea	Total viable counts	Enterobacte- riacea	Total viable counts
Control	5.29±0.01 <sup>f</sup>	5.48±0.01 <sup>c</sup>	5.28±0.05 <sup>f</sup>	5.40± 0.02 <sup>f</sup>	5.28± 0.00 <sup>e</sup>	5.41±0.01 <sup>g</sup>
OB2	5.02±0.02 <sup>e</sup>	5.11±0.03 <sup>a</sup>	4.88±0.00 <sup>d</sup>	5.28± 0.01 <sup>e</sup>	5.00± 0.04 <sup>d</sup>	5.19±0.03 <sup>f</sup>
OB3	4.70±0.05 <sup>c</sup>	5.08±0.00 <sup>a</sup>	4.88±0.07 <sup>d</sup>	5.16± 0.00 <sup>d</sup>	5.00± 0.02 <sup>d</sup>	5.12± 0.02 <sup>e</sup>
OB4	4.60± 0.02 <sup>b</sup>	5.50± 0.00 <sup>c</sup>	4.78± 0.04 <sup>c</sup>	4.93± 0.03 <sup>b</sup>	4.70± 0.00 <sup>c</sup>	4.90± 0.06 <sup>c</sup>
OB5	4.40± 0.03 <sup>a</sup>	5.56± 0.02 <sup>c</sup>	4.18± 0.02 <sup>a</sup>	4.74± 0.02 <sup>a</sup>	4.54±0.04 <sup>b</sup>	4.70± 0.02 <sup>ab</sup>
WL6	5.02± 0.01 <sup>e</sup>	5.22± 0.01 <sup>b</sup>	5.04± 0.01 <sup>e</sup>	5.06± 0.04 <sup>c</sup>	5.00± 0.07 <sup>d</sup>	5.04± 0.01 <sup>d</sup>
WL7	4.78± 0.01 <sup>d</sup>	5.11± 0.03 <sup>a</sup>	5.04± 0.05 <sup>e</sup>	5.04± 0.10 <sup>c</sup>	4.98± .0.08 <sup>d</sup>	5.02± 0.00 <sup>d</sup>
WL8	4.60± 0.02 <sup>b</sup>	5.26± 0.01 <sup>b</sup>	4.65± 0.03 <sup>b</sup>	4.95± 0.03 <sup>b</sup>	4.54± 0.02 <sup>b</sup>	4.78± 0.00 <sup>b</sup>
WL9	4.54± 0.05 <sup>b</sup>	5.56± 0.00 <sup>c</sup>	4.18± 0.02 <sup>a</sup>	4.78± 0.00 <sup>a</sup>	4.40± 0.04 <sup>a</sup>	4.65±0.02 <sup>a</sup>

**Key:** Mean followed by the same letter is not significantly different (p > 0.05)

**Table 2a:** Enterobacteriaceae and total viable counts (log 10CFU/g) of *Clarias gariepinus* treated with onion bulb

Treatment	Fish sites	4 Weeks		8 Weeks		12 Weeks	
		Enterobac- teriacea	Total viable counts	Enterobacte- riacea	Total viable counts	Enterobacte- riacea	Total viable counts
Control	Skin	4.04± 0.02 <sup>g</sup>	4.11± 0.10 <sup>g</sup>	4.02± 0.00 <sup>f</sup>	4.10± 0.02 <sup>h</sup>	3.71± 0.00 <sup>g</sup>	3.74± 0.02 <sup>g</sup>
	Liver	3.88± 0.04 <sup>g</sup>	3.89±0.00 <sup>g</sup>	3.85± 0.02 <sup>g</sup>	3.87± 0.01 <sup>h</sup>	3.65± 0.02 <sup>g</sup>	3.70± 0.04 <sup>f</sup>
	Gill	3.97± .002 <sup>g</sup>	4.09± 0.04 <sup>g</sup>	3.93± 0.01 <sup>f</sup>	4.06± 0.01 <sup>g</sup>	3.46± 0.01 <sup>h</sup>	3.49± 0.02 <sup>g</sup>
	Intestine	3.85± 0.00 <sup>h</sup>	3.85± 0.05 <sup>a</sup>	3.79± 0.00 <sup>f</sup>	3.82± 0.03 <sup>g</sup>	3.52±0.02 <sup>f</sup>	3.58± 0.06 <sup>f</sup>
OB2	Skin	3.76± 0.00 <sup>f</sup>	3.91± 0.07 <sup>f</sup>	3.72± 0.00 <sup>e</sup>	3.89± 0.01 <sup>g</sup>	3.58± 0.09 <sup>f</sup>	3.70± 0.02 <sup>f</sup>
	Liver	3.80±0.02 <sup>f</sup>	3.80± 0.06 <sup>f</sup>	3.74± 0.05 <sup>f</sup>	3.77± 0.00 <sup>g</sup>	3.62±0.10 <sup>e</sup>	3.66± 0.05 <sup>ef</sup>
	Gill	3.72± 0.02 <sup>f</sup>	3.92± 0.02 <sup>f</sup>	3.68± 0.02 <sup>e</sup>	3.91± 0.05 <sup>f</sup>	3.24± 0.01 <sup>h</sup>	3.46± 0.03 <sup>f</sup>
	Intestine	3.81± 0.01 <sup>g</sup>	3.77± 0.00 <sup>a</sup>	3.77± 0.04 <sup>f</sup>	3.74± 0.00 <sup>f</sup>	3.42±0.04 <sup>e</sup>	3.56± 0.01 <sup>ef</sup>
OB3	Skin	3.65±0.01 <sup>e</sup>	3.76±0.00 <sup>e</sup>	3.59± 0.01 <sup>d</sup>	3.73± 0.00 <sup>f</sup>	3.49± 0.03 <sup>e</sup>	3.62± 0.00 <sup>e</sup>
	Liver	3.60±0.02 <sup>e</sup>	3.67±0.08 <sup>d</sup>	3.54± 0.01 <sup>e</sup>	3.71± 0.00 <sup>f</sup>	3.43± 0.02 <sup>d</sup>	3.62± 0.01 <sup>e</sup>
	Gill	3.62±0.00 <sup>e</sup>	3.64±0.08 <sup>d</sup>	3.58±0.00 <sup>d</sup>	3.62± 0.01 <sup>d</sup>	3.11± 0.04 <sup>g</sup>	3.44± 0.01 <sup>e</sup>
	Intestine	3.65± 9.09 <sup>f</sup>	3.69±0.01 <sup>a</sup>	3.61± 0.02 <sup>e</sup>	3.68± 0.03 <sup>e</sup>	3.20± 0.09 <sup>d</sup>	3.40± 0.05 <sup>d</sup>
OB4	Skin	3.53± 0.00 <sup>d</sup>	3.63±0.01 <sup>d</sup>	3.48± 0.05 <sup>c</sup>	3.59±0.04 <sup>e</sup>	3.41± 0.09 <sup>d</sup>	3.56± 0.04 <sup>d</sup>
	Liver	3.51±0.05 <sup>d</sup>	3.62±0.01 <sup>c</sup>	3.46±0.02 <sup>d</sup>	3.57±0.00 <sup>d</sup>	3.32± 0.07 <sup>d</sup>	3.52± 0.03 <sup>d</sup>
	Gill	3.52±0.06 <sup>d</sup>	3.61±0.02 <sup>d</sup>	3.42±0.02 <sup>c</sup>	3.59± 0.01 <sup>d</sup>	3.09± 0.01 <sup>e</sup>	3.28± 0.00 <sup>c</sup>
	Intestine	3.54±0.01 <sup>e</sup>	3.69±0.01 <sup>a</sup>	3.50±0.01 <sup>d</sup>	3.64± 0.02 <sup>e</sup>	3.13± 0.00 <sup>c</sup>	3.39± 0.05 <sup>d</sup>
OB5	Skin	3.48± 0.07 <sup>c</sup>	3.49± 0.02 <sup>b</sup>	3.39±0.04 <sup>b</sup>	3.45± 0.03 <sup>b</sup>	3.18±0.06 <sup>b</sup>	3.46± 0.08 <sup>b</sup>
	Liver	3.46± 0.09 <sup>c</sup>	3.53± 0.02 <sup>b</sup>	3.34±0.03 <sup>c</sup>	3.53± 0.13 <sup>c</sup>	3.28±0.05 <sup>b</sup>	3.41± 0.00 <sup>c</sup>
	Gill	3.40± 0.02 <sup>c</sup>	3.53±0.03 <sup>c</sup>	3.33±0.01 <sup>b</sup>	3.51± 0.02 <sup>c</sup>	2.81± 0.00 <sup>d</sup>	3.24± 0.02 <sup>b</sup>
	Intestine	3.48±0.06 <sup>d</sup>	3.54±0.00 <sup>a</sup>	3.42± 0.05 <sup>c</sup>	3.52± 0.01 <sup>d</sup>	3.06± 0.10 <sup>b</sup>	3.31± 0.01 <sup>c</sup>

**Key:** Mean followed by the same letter is not significantly different (p > 0.05)

**Table 2b:** Enterobacteriaceae and total viable counts (log 10CFU/g) of *Clarias gariepinus* treated with walnut leaf

Treatment	Fish sites	4 Weeks		8 Weeks		12 Weeks	
		Enterobacte- riacea	Total viable counts	Enterobac- teriacea	Total viable counts	Enterobac- teriacea	Total viable counts
Control	Skin	4.04± 0.02 <sup>g</sup>	4.11± 0.10 <sup>g</sup>	4.02± 0.00 <sup>f</sup>	4.10± 0.02 <sup>h</sup>	3.71± 0.00 <sup>g</sup>	3.74± 0.02 <sup>g</sup>
	Liver	3.88± 0.04 <sup>g</sup>	3.89±0.00 <sup>g</sup>	3.85± 0.02 <sup>g</sup>	3.87± 0.01 <sup>h</sup>	3.65± 0.02 <sup>g</sup>	3.70± 0.04 <sup>f</sup>
	Gill	3.97± .002 <sup>g</sup>	4.09± 0.04 <sup>g</sup>	3.93± 0.01 <sup>f</sup>	4.06± 0.01 <sup>g</sup>	3.46± 0.01 <sup>h</sup>	3.49± 0.02 <sup>g</sup>
	Intestine	3.85± 0.00 <sup>h</sup>	3.85± 0.05 <sup>a</sup>	3.79± 0.00 <sup>f</sup>	3.82± 0.03 <sup>g</sup>	3.52±0.02 <sup>f</sup>	3.58± 0.06 <sup>f</sup>
WL6							
	Skin	3.54±0.01 <sup>d</sup>	3.61± 0.00 <sup>d</sup>	3.48±0.02 <sup>c</sup>	3.59±0.07 <sup>e</sup>	3.51±0.04 <sup>e</sup>	3.63±0.08 <sup>e</sup>
	Liver	3.48±0.00 <sup>cd</sup>	3.70± 0.05 <sup>e</sup>	3.43±0.06 <sup>d</sup>	3.66±0.08 <sup>e</sup>	3.39±0.01 <sup>f</sup>	3.57±0.05 <sup>g</sup>
	Gill	3.60± 0.01 <sup>e</sup>	3.69± 0.07 <sup>e</sup>	3.57±0.04 <sup>d</sup>	3.66±0.00 <sup>e</sup>	3.31±0.03 <sup>f</sup>	3.56±0.03 <sup>d</sup>
	Intestine	3.48± 0.02 <sup>d</sup>	3.53± 0.03 <sup>a</sup>	3.45±0.01 <sup>cd</sup>	3.48±0.02 <sup>c</sup>	3.39±0.09 <sup>e</sup>	3.50±0.01 <sup>e</sup>
WL7							
	Skin	3.46± 0.05 <sup>bc</sup>	3.53± 0.08 <sup>c</sup>	3.41±0.01 <sup>b</sup>	3.50±0.02 <sup>c</sup>	3.32±0.02 <sup>c</sup>	3.51±0.02 <sup>c</sup>
	Liver	3.36± 0.03 <sup>b</sup>	3.53±0.00 <sup>b</sup>	3.29±0.02 <sup>b</sup>	3.48±0.01 <sup>b</sup>	3.11±0.07 <sup>c</sup>	3.38±0.05 <sup>d</sup>
	Gill	3.44± 0.00 <sup>c</sup>	3.57± 0.01 <sup>c</sup>	3.41±0.09 <sup>c</sup>	3.53±0.07 <sup>c</sup>	3.06±0.00 <sup>c</sup>	3.31±0.01 <sup>b</sup>
	Intestine	3.37± 0.04 <sup>c</sup>	3.50± 0.02 <sup>a</sup>	3.31±0.00 <sup>b</sup>	3.46±0.02 <sup>bc</sup>	3.22±0.02 <sup>d</sup>	3.28±0.09 <sup>bc</sup>
WL8							
	Skin	3.43± 0.03 <sup>b</sup>	3.43± 0.01 <sup>a</sup>	3.41±0.03 <sup>b</sup>	3.38±0.06 <sup>a</sup>	3.11±0.01 <sup>b</sup>	3.32±0.02 <sup>a</sup>
	Liver	3.35± 0.00 <sup>b</sup>	3.51± 0.00 <sup>a</sup>	3.36±0.01 <sup>c</sup>	3.46±0.05 <sup>b</sup>	2.93±0.02 <sup>a</sup>	3.31±0.08 <sup>b</sup>
	Gill	3.30± 0.01 <sup>b</sup>	3.46± 0.04 <sup>b</sup>	3.33±0.01 <sup>b</sup>	3.42±0.04 <sup>b</sup>	2.70±0.05 <sup>b</sup>	3.11±0.03 <sup>a</sup>
	Intestine	3.43± 0.01 <sup>b</sup>	3.40± 0.02 <sup>a</sup>	3.24±0.02 <sup>b</sup>	3.37±0.01 <sup>a</sup>	3.11±0.02 <sup>c</sup>	3.26±0.06 <sup>ab</sup>
WL9							
	Skin	3.35± 0.07 <sup>a</sup>	3.42± 0.01 <sup>a</sup>	3.33±0.02 <sup>a</sup>	3.53±0.09 <sup>d</sup>	2.90±0.01 <sup>a</sup>	3.29±0.06 <sup>a</sup>
	Liver	3.20± 0.08 <sup>a</sup>	3.35± 0.09 <sup>a</sup>	3.16±0.05 <sup>a</sup>	3.33±0.04 <sup>a</sup>	2.88±0.01 <sup>a</sup>	3.28±0.04 <sup>a</sup>
	Gill	3.04± 0.01 <sup>a</sup>	3.15± 0.00 <sup>a</sup>	2.93±0.07 <sup>a</sup>	3.06±0.03 <sup>a</sup>	2.70±0.03 <sup>a</sup>	3.06±0.02 <sup>a</sup>
	Intestine	3.18± 0.03 <sup>a</sup>	3.37±0.03 <sup>a</sup>	3.11±0.02 <sup>a</sup>	3.45±0.13 <sup>b</sup>	2.98±0.04 <sup>a</sup>	3.23±0.00 <sup>a</sup>

**Key:** Mean followed by the same letter is not significantly different ( $p > 0.05$ )

**Table 3:** Organ index of *Clarias gariepinus* treated with onion bulb and walnut leaf residues

Treatments	Liver	Spleen	Kidney	Heart
Control	0.007±0.00 <sup>ab</sup>	0.002±0.01 <sup>a</sup>	0.004±0.00 <sup>ab</sup>	0.002±0.01 <sup>a</sup>
OB2	0.008±0.01 <sup>ab</sup>	0.002±0.01 <sup>a</sup>	0.003±0.02 <sup>a</sup>	0.002±0.00 <sup>a</sup>
OB3	0.011±0.00 <sup>b</sup>	0.002±0.00 <sup>a</sup>	0.002±0.02 <sup>a</sup>	0.002±0.00 <sup>a</sup>
OB4	0.004±0.01 <sup>a</sup>	0.001±0.02 <sup>a</sup>	0.002±0.00 <sup>a</sup>	0.002±0.02 <sup>a</sup>
OB5	0.009±0.02 <sup>b</sup>	0.003±0.00 <sup>a</sup>	0.003±0.01 <sup>a</sup>	0.003±0.00 <sup>a</sup>
WL6	0.009±0.01 <sup>b</sup>	0.002±0.01 <sup>a</sup>	0.007±0.00 <sup>b</sup>	0.002±0.01 <sup>a</sup>
WL7	0.008±0.00 <sup>ab</sup>	0.002±0.00 <sup>a</sup>	0.005±0.00 <sup>ab</sup>	0.002±0.01 <sup>a</sup>
WL8	0.007±0.01 <sup>ab</sup>	0.002±0.01 <sup>a</sup>	0.004±0.01 <sup>ab</sup>	0.002±0.00 <sup>a</sup>
WL9	0.007±0.00 <sup>ab</sup>	0.002±0.01 <sup>a</sup>	0.005±0.01 <sup>ab</sup>	0.002±0.01 <sup>a</sup>

**Key:** Mean followed by the same letter is not significantly different ( $p > 0.05$ )

## Statistical Analysis

Bacteriological characteristics and organ indices resulting from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006 version 15.0). Duncan's multiple range test was used to compare differences among individual means.

## Results

### Microbiological Analyses of Water and *Clarias Gariepinus*

The results of Enterobacteriaceae and total viable counts of water samples and *Clarias gariepinus* (skin, liver, intestine and gills) fed diets containing onion bulb and walnut leaf residues are presented on Tables 1 and 2.

### Organs Index of *Clarias Gariepinus*

The results of organ index were presented in Table 3.

## Discussion

Results of these findings show that enterobacteriaceae in water was higher in the control than the treated groups fed diets containing onion bulb and walnut leaf residues. The values decreased in all the treated groups with increasing inclusions of the residues in the diets. The control diet recorded highest enterobacteriaceae for 4 weeks, 8 weeks and 12 weeks. The lowest enterobacteriaceae was recorded in OB5 in onion bulb residue treatments and WL9 in walnut leaf residue treatments for 4 weeks, 8 weeks and 12 weeks.

Total viable count (TVC) from the water of *Clarias gariepinus* fed onion bulb and walnut leaves showed that the TVC was higher in the control diets at 4 weeks, 8 weeks and 12 weeks and the lowest TVC was recorded in OB5 in onion bulb residue treatments and WL9 in walnut leaf residue treatments for water at 4 weeks, 8 weeks and 12 weeks. The values of TVC obtained from the present findings decreased in all the treated groups with increasing inclusion levels of the residues.

Results of enterobacteriaceae from this study revealed that bacteria load on the water of the experimental tanks was more affected

by *A. cepa* and *T. conophorum* than the control. Also, the enterobacteriaceae and total viable count in water sample for 4, 8 and 12 weeks were significantly different ( $P < 0.05$ ) from the control. The findings of this study agree with the work of Shalaby *et al.*, (2006) who obtained decreases in bacterial load of water fed *O. niloticus* on garlic and chloramphenicol at different graded levels. The report of Sugita *et al.*, (1989) was in agreement with the present work. However, these results contradict those of Al-Harbi and Uddin (2003) who reported that counts of total viable bacteria were in the range of  $3.74 - 3.38 \log_{10}$  CFU/ml in pond water without any treatment; these values were lower than enterobacteriaceae in the control of this present work. Also, Nedoluha and Westhoff, (1997) reported  $6.80 \log_{10}$  CFU/ml for fish growing water in tanks with a stocking density of 3g fish/l; these values were higher than the one obtained in this present study.

However, the results of total viable count (TVC) from water with *C. gariepinus* fed diets with onion bulb and walnut leaf residues were lower than the water from the control. The TVC in this present study was lower than that reported by McKeon *et al.*, (2001) in pre-filtered water of recirculating systems ( $106 \text{ CFU}/100\text{ml}$ ), but in filtered water it was  $4.20 \log_{10} \text{ CFU}/100\text{ml}$  which also agreed with result obtained in 8 weeks and 12 weeks of the study.

The antimicrobial effect of walnut leaf and onion bulb residues in diets led to reductions in the microbial load of water of experimental tanks and inhibited the growth of microorganisms or pathogenic bacteria that result in infection of fish. Enterobacteriaceae in skin, liver, gills and intestine of *C. gariepinus* on control diet were higher than the treated groups. The lowest enterobacteriaceae was recorded in OB5 in onion bulb residue treatments for skin, gill, liver and intestine at 4 weeks, 8 weeks and 12 weeks and WL9 in walnut leaf residue treatments for skin, gill, liver and intestine at 4 weeks, 8 weeks and 12 weeks. The values decreased in treated groups as the level of inclusion (0.5%, 1.0%, 1.5% and 2.0%) increased and as the months increased.

Moreover, the result of TVC in skin, liver, gills and intestine of *C. gariepinus* of the control was higher than the onion bulb

and walnut leaf residues and TVC in skin, gill, liver and intestine was highest in the control diets at 4 weeks, 8 weeks and 12 weeks. The lowest TVC was recorded in OB5 in onion bulb residue treatments in skin, gill, liver and intestine at 4 weeks, 8 weeks and 12 weeks while the lowest TVC was recorded in WL9 in walnut leaf residue treatments in skin, gill, liver and intestine at 4 weeks, 8 weeks and 12 weeks.

The values decreased in treated groups as the levels of inclusion (0.5%, 1.0%, 1.5% and 2.0%) increased and as the months increased. The findings of enterobacteriaceae from this study revealed that bacterial load in skin, liver, gills and intestine of *C. gariepinus* fed *A. cepa* and *T. conophorum* were lower than the control. Enterobacteriaceae and total viable count of (skin, liver, gill and intestine) for 4, 8 and 12 weeks were significantly lower ( $P < 0.05$ ) than the control. The reason for this might be due to the presence of antimicrobial properties present in walnut leaf and onion bulb. Treatment with *T. conophorum* was more effective in reducing bacteria in skin, liver, gills and intestine. The findings of this study are in agreement with the work of Shalaby et al., (2006) where there were low values in muscles and intestine of *O. niloticus* fed *Allium sativum* and chloramphenicol on different graded levels. Also, Shalaby et al., (2006) revealed that coliform count from the intestine of fish fed garlic diet was 4.78 – 5.69 log<sub>10</sub> CFU/g and in fish fed on chloramphenicol diet was 3.48 – 5.45 log<sub>10</sub> CFU/g this report was in agreement with the present findings.

Organ indices showed that the liver, heart, kidney and spleen i.e. the hepatosomatic and splenosomatic indices were not significantly increased in all the treated groups. This finding agrees with the report of Azza and Abd-El-Rhman, (2009). Fox et al., (1997) reported that the organosomatic indices are indicators of health (hepatosomatic index and splenosomatic index) which could be used to predict the health status of fish. The findings showed no traces of oedema and high variation of the intestinal organs, the inclusion of walnut leaf and onion bulb in the diet of *C. gariepinus* could therefore, be considered safe and non-toxic for consumption.

In conclusion, since antimicrobial effects of walnut leaf and onion bulb residue resulted in reduction in microbial loads of water and fish the inclusion of these plants as a replacement or additive in fish feed could aid productivity in aquaculture industry. Their use in aquaculture industry is safe since they are highly biodegradable and do not have any side effects (Blumenthal et al., 2000) such as drug resistance that have been generally reported in synthetic antibiotics.

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## EFFECT OF AGE ON BLOOD BIOCHEMICAL PROFILES OF THE AFRICAN BLACK OSTRICH IN GHANA

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### Abstract

The objective of this study was to evaluate the effect of age on blood biochemical indices in the African Black Ostrich. Blood samples were collected from 56, 60 and 64-week old ostriches (8 birds per age group) from a private farm and the blood biochemical profiles in the plasma of the birds determined. The 64 week-old ostriches had higher cholesterol levels than the 56 and 60 week old ostriches. The overall mean cholesterol level was 1.81 mmol/L. Triglyceride levels were significantly ( $P < 0.05$ ) influenced by age with the 64 week-old ostriches having higher values than 60 week-old ostriches (0.64 mmol/L versus 0.48 mmol/L). The overall mean triglyceride concentration was 0.55 mmol/L. The other biochemical parameters measured were not significantly ( $P > 0.05$ ) affected by age. Results from this study demonstrate that age influences the productivity and health of the African Black Ostrich in Ghana.

**Keywords:** Age, African Black Ostrich, Biochemical profile

## EFFET DE L'AGE SUR LES PROFILS BIOCHIMIQUES SANGUINS DE L'AUTRUCHE NOIRE D'AFRIQUE AU GHANA

### Résumé

L'objectif de cette étude était d'évaluer l'effet de l'âge sur les indices biochimiques sanguins de l'autruche noire d'Afrique. Des échantillons de sang ont été prélevés sur des autruches âgées de 56, 60 et 64 semaines (8 oiseaux par groupe d'âge) dans une ferme privée, et les profils biochimiques du sang dans le plasma de ces oiseaux ont été déterminés. Les autruches âgées de 64 semaines avaient des taux de cholestérol plus élevés que celles âgées de 56 et 60 semaines. Le taux de cholestérol total moyen était de 1,81 mmol / L. Les taux de triglycérides étaient significativement ( $P < 0,05$ ) influencés par l'âge, les autruches âgées de 64 semaines ayant des valeurs supérieures à celles de 60 semaines (0,64 mmol / L contre 0,48 mmol / L). La concentration totale moyenne de triglycérides était de 0,55 mmol / L. Les autres paramètres biochimiques mesurés n'étaient pas significativement ( $P > 0,05$ ) affectés par l'âge. Les résultats de cette étude démontrent que l'âge influence la productivité et la santé de l'autruche noire d'Afrique au Ghana.

**Mots-clés:** Age ; Autruche noire d'Afrique ; Profil biochimique

## Introduction

Determination of nutritional and physiological conditions of birds using biochemical parameters is useful in understanding ecological and behavioural problems (Ferrer, 1990). Biochemical indices are very important in assessing infection, organ function and many diseases in animals by comparing the levels of haematological and biochemical indices in the blood with reference values (Raukar *et al.*, 2007). These biochemical indices are influenced by factors such as the physiological state of the bird, species, age, sex, nutritional status, seasonal changes and conditions in particular geographic areas (Ferrer, 1990; Kaneko *et al.*, 1997; Campbell, 2004; Erenier *al.*, 2006; Raukar *et al.*, 2007).

In Ghana, a number of individuals have recently gone into ostrich farming because of the high biological value of ostrich meat and its high returns in terms of income. Although haematological and biochemical profiles have been studied and reference values established for ostrich from some regions of the world (Palomeque *et al.*, 1991; Mushi *et al.*, 1999; Levy *et al.*, 1989; Verstappen *et al.*, 2002; Raukar and Simpraga, 2005), no such studies have been conducted in Ghana. Blood chemistry profiles are extremely important in the health management of animals. This study therefore was aimed at determining baseline values for several parameters, based on age in apparently healthy ostriches raised in Ghana. This may provide better understanding of the physiology and adaptation of the species to environmental conditions as well as improve its performance. This would eventually contribute to the success of ostrich farming in Ghana.

## Materials and Methods

### *Animals and location*

Twenty four African Black ostriches (*Struthiocamelus*) of both sexes raised on a private farm located at Nsawam – GyaTsui Newtown (latitude 05° 48'N and longitude 00° 21'W) in the Akuapim South district were used in the study. The area is situated within the semi-deciduous forest zone of Ghana. The birds were kept in a semi-intensive system

of management and were kept under natural conditions of light and temperature and supplied with water *ad libitum* and fed a ration once a day. The birds were in three age groups (56, 60 and 64 weeks). Eight birds from each age group made up of 7 hens and 1 cock: 56 weeks; 7 hens and 1 cock: 60 weeks; 5 hens and 3 cocks: 64 weeks) formed the 3 treatment groups.

### *Blood samples*

Blood was drawn from the jugular vein between 09:00 and 12:00 before feeding to reduce changes in the levels of blood constituents associated with nutrient absorption. Each bird was manually caught by hand and restrained. A woollen sock was placed over the head to avoid stress and for ease of containment (Levy *et al.*, 1989; Polat *et al.*, 2003). The blood was drawn into heparinised 10ml vacutainer blood collection tubes. The samples were placed on ice after collection and sent to the laboratory immediately for analysis. Blood was centrifuged at 5000rpm for 5 minutes at 4°C and the plasma obtained was analysed for concentrations of glucose, total protein, albumin, triglyceride, cholesterol, urea, creatinine, total bilirubin, and enzymatic activities of Alanine-Aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma-glutamyltransferase (GGT) and Alkaline Phosphatase (ALP) using a biochemical analyzer. Sodium and inorganic potassium were also determined using the Sodium Potassium auto analyser (BiolaboDiagnostics, Kenza Biochemistry, France).

## Results

The effect of age on biochemical variables in 56, 60 and 64 week old ostriches are shown in Table I. Age did not significantly ( $P > 0.05$ ) affect glucose, total protein, albumin, total bilirubin, urea and creatinine levels. The overall mean values reported for the above mentioned blood constituents were 9.24 mmol/L, 52.08 g/L, 24.37 g/L, 4.87  $\mu$ mol, 1.25 mmol/L and 35.9 mmol/L, respectively. Glucose and urea levels tended to increase with age whilst total protein and creatinine levels decreased with age but these influences were not significant ( $P > 0.05$ ).

**Table 1:** Effect of age (mean  $\pm$  SE) on levels of blood biochemical variables in African Black Ostriches

Blood parameters	Age (weeks)			Mean
	56 (n = 8)	60 (n = 8)	64 (n = 8)	
Glucose (mmol/L)	8.81 $\pm$ 0.3	9.31 $\pm$ 0.3	9.59 $\pm$ 0.3	9.24
Cholesterol (mmol/L)	1.78b $\pm$ 0.1	1.53b $\pm$ 0.1	2.10a $\pm$ 0.1	1.81
Triglycerides (mmol/L)	0.55ab $\pm$ 0.04	0.48b $\pm$ 0.04	0.64a $\pm$ 0.04	0.55
Total Protein (g/L)	53.63 $\pm$ 3.5	52.00 $\pm$ 3.5	50.62 $\pm$ 3.5	52.08
Albumin (g/L)	23.38 $\pm$ 1.3	25.63 $\pm$ 1.3	24.13 $\pm$ 1.3	24.37
Total Bilirubin( $\mu$ mol/L)	4.23 $\pm$ 0.8	3.96 $\pm$ 0.8	6.43 $\pm$ 0.8	4.87
Urea (mmol/L)	1.17 $\pm$ 0.1	1.20 $\pm$ 0.1	1.37 $\pm$ 0.1	1.25
Creatinine (mmol/L)	38.83 $\pm$ 2.6	35.88 $\pm$ 2.6	33.00 $\pm$ 2.6	35.9
ALT (U/L)	29.75 $\pm$ 2.7	29.13 $\pm$ 2.7	26.50 $\pm$ 2.7	28.46
AST (U/L)	252.63 $\pm$ 10.7	263.25 $\pm$ 10.7	256.13 $\pm$ 10.1	257.3
GGT (U/L)	8.50 $\pm$ 1.9	14.50 $\pm$ 1.9	12.75 $\pm$ 1.9	11.92
ALP (U/L)	47.00 $\pm$ 4.2	43.00 $\pm$ 4.2	46.63 $\pm$ 4.2	45.54
Sodium (mmol/L)	142.88 $\pm$ 1.1	140.50 $\pm$ 1.1	142.00 $\pm$ 1.1	141.79
Potassium(mmol/L)	4.26 $\pm$ 0.1	4.26 $\pm$ 0.1	4.68 $\pm$ 0.1	4.4

Means on the same row bearing different superscripts (a, b) are significantly ( $P < 0.05$ ) different

Age was a significant ( $P < 0.05$ ) source of variation in cholesterol levels. The 64 week-old ostriches had higher cholesterol levels than the 56 and 60 week old ostriches. The overall mean cholesterol level was 1.81 mmol/L. Triglyceride levels were significantly ( $P < 0.05$ ) influenced by age with the 64 week-old ostriches having higher values than 60 week-old ostriches (0.64 mmol/L versus 0.48 mmol/L). The cholesterol levels for the 56 week-old ostriches were similar for the 60 and 64 week- olds. The overall mean triglyceride concentration was 0.55 mmol/L.

The concentration of enzymes and minerals measured were not significantly ( $P > 0.05$ ) affected by age. The overall means recorded were 28.46 U/L, 257.3 U/L, 11.92 U/L, 45.54 U/L, 141.79 mmol/L and 4.4 mmol/L for ALT, AST, GGT, ALP Sodium and potassium, respectively.

## Discussion

Normal blood glucose values in the ostrich have been reported to range from 9.87 to 14.62 mmol/L (Palomeque *et al.*, 1991; Verstappen *et al.*, 2002; Moniello *et al.*, 2005). The values obtained in the present study was

comparable to the minimum values in this physiological range. Although age had no effect on glucose concentration, the absolute values tended to increase with age, with the 64 week old ostriches having the highest value of 9.59 mmol/L and the 54 week olds having the lowest value of 8.81 mmol/L. This trend supports earlier findings by other researchers (Levy *et al.*, 1989; Moniello *et al.*, 2005).

The overall mean cholesterol level was 1.81 mmol/L. This was comparable to the values 1.58 mmol/L and 1.75 mmol/L reported by Mushi *et al.*, (1998) and Moniello *et al.*, (2005) respectively but lower than the value 2.80 mmol/L and 3.0 mmol/L reported by Levy *et al.*, (1989) and Palomeque *et al.*, (1991), respectively. The variation may be due to the fat or protein content of the diet, since high levels of fat and or low level of protein result in high cholesterol levels (Palomeque *et al.*, 1991). The diet of the ostriches in the study conducted by Palomeque *et al.*, (1991) consisted of fresh vegetables and vitamin supplements whiles Levy *et al.*, (1989) fed chopped alfalfa or peanut hay, supplemented with minerals and vitamins in a concentrate normally prepared for turkeys. Variations in genetic factors are also known to cause to differences in cholesterol levels.

This study used the African Black ostriches which may have accounted for the difference in cholesterol levels obtained for the Massai ostriches used by Levy *et al.*, (1989) and Palomeque *et al.*, (1991).

Age affected triglyceride levels. Ostriches aged 64 weeks had higher triglyceride levels than those aged 60 weeks. The overall mean value recorded for triglycerides was 0.55 mmol/L. Results obtained in this study were lower than 1.20 mmol/L, 1.32 mmol/L and 1.09 mmol/L reported by Levy *et al.*, (1989), Mushi *et al.*, (1998) and Moniello *et al.*, (2005), respectively. The differences in nutritional status of the ostriches and the different management conditions under which they were raised may account for the differences in the triglyceride levels as suggested by Haq *et al.*, (1999).

Total protein concentration in this study was not influenced by age. Normal total protein values in the ostrich range from 30 to 56 g/L (Levy *et al.*, 1989; Palomeque *et al.*, 1991; Mushi *et al.*, 1998; Verstappen *et al.*, 2002). The overall mean value of 52.08g/L obtained in the present study was within this normal range. According to Palomeque *et al.*, (1991), high protein in younger birds is due to higher metabolic rates in relation to rapid tissue and feather growth and the high demand for protein. Albumin was not influenced by age of the ostriches in this study. Earlier studies indicate that albumin level varies with nutrition, hormonal balance and stress (Thrall *et al.*, 2004). The latter seems to be the case in this study as the birds experiment stressful chasing during sample collection. The overall mean albumin level of 24.37 g/L was comparatively higher than those reported from other studies. Mushi *et al.*, (1998) found albumin level of 22.14 g/L and cited figures of 20.4 g/L (van Heerden *et al.*, 1985) and 18.00 g/L.

Total bilirubin concentration was not influenced by age. The overall mean recorded was 4.87  $\mu\text{mol/L}$  with the highest value of 6.43  $\mu\text{mol/L}$  found in the birds aged 64 weeks and the lowest value of 3.96  $\mu\text{mol/L}$  in those aged 60 weeks. Total bilirubin levels in the ostriches aged 56 and 60 weeks were comparable to the mean value (3.96  $\mu\text{mol/L}$ ) reported by Mushi *et al.*, (1998). The total bilirubin value in 60 weeks-old ostriches in this study was however

lower than those reported by Moniello *et al.*, (2005) in 12 and 24 months old ostriches. The reported values were 6.80  $\mu\text{mol/L}$  and 9.36  $\mu\text{mol/L}$ , respectively.

The overall mean urea concentration was 1.25 mmol/L. This result is higher than the value 0.40 mmol/L reported in other studies (van Heerden *et al.*, 1985; Levy *et al.*, 1989; Mushi *et al.*, 1998), but lower than the finding of Palomeque *et al.*, (1991). The high urea level observed in this study may be attributable to the relatively higher level of crude protein (18.56%) in the diet of the ostrich, as protein catabolism is known to influence urea level.

Creatinine levels are influenced by a number of factors including muscle mass. It increases with age when the muscular tissue turnover accelerates. The creatinine levels were similar in all the age groups examined in this study. It could therefore be assumed that in the growing ostriches changes in muscle mass may have reduced already at 56 weeks. An overall mean of 35.9 mmol/L was obtained. This value, however, was higher than the values of 28 mmol/L, 27 mmol/L and 23.7 mmol/L reported by Levy *et al.*, (1989), Mushi *et al.*, (1998) and Moniello *et al.*, (2005). This may be due to the fact that birds in the present study had to be chased for sometime before capture for blood sampling. Quintavalla *et al.*, (2001) had reported that serum values of creatinine could increase following physical exercise, such as that associated with capture of animals for blood sampling.

Enzymatic activity of various organs causes biochemical changes in these organs and these activities increase with age up to 14 months in ostriches (Moniello *et al.*, 2005). Age did not influence the levels of any of the enzymes measured in the plasma. The birds in the present study were between 14 and 16 months of age hence the insignificant differences in the levels of the enzymes measured may be due to the age differences being only few weeks. ALT in the blood could be attributed to the physiological activities of the different systems in the body and this result in high or low ALT values (Moniello *et al.*, 2005). A high level of GGT is an indication of hepatic or muscular pathologies. According to Moniello *et al.*, (2005), standard values of GGT for the

ostrich had not yet been defined. Plasma sodium and potassium levels in the ostrich were not influenced by age.

### Conclusion

The results of this study indicated that age had a significant effect on total cholesterol and triglycerides levels but not the other parameters examined. Cholesterol and triglyceride concentrations were higher in the 64-week old ostriches than the 60 and 56 week olds in the African Black Ostrich.

Most of the levels of blood constituents recorded (glucose, total protein, cholesterol, AST, Sodium and Potassium) either compared favourably with or fell within the normal ranges reported for ostriches.

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This study will provide understanding of the physiology and adaptation of the species to environmental conditions as well as improve its performance.

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## PREVALENCE OF HYDATIDOSIS IN CAMELS SLAUGHTERED IN SOKOTO CENTRAL ABATTOIR, SOKOTO, NIGERIA

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### Abstract

Data on the prevalence of hydatid cyst in camels slaughtered at the metropolitan abattoir in Sokoto, Nigeria, were collected based on post-mortem inspection over a period of 2 months. Camels of different sexes (male and female) and age categories (puberty, < 3 years of age; and beyond the age of puberty, ≥ 3 years of age) were examined at post-mortem. Out of the 200 slaughtered camels examined, 84 (42%) were found to be infected. The prevalence of infection in camels < 3 years of age (not yet at puberty) was 39.13%, while that of ≥ 3 years of age (beyond the age of puberty) was 42.37%. Out of the 84 (42%) camels infected, 78 (92.86%) of the infections were in the lungs, 21 (25.0%) in the liver and 10 (11.90%) in the spleen. There was no statistical association between the sex and the hydatid cyst disease; and between the different age categories and the hydatid cyst disease ( $P > 0.05$ ). Zoonotic importance of the disease and suggested control measures has been discussed.

**Key words:** Hydatidosis, Slaughter, Camel, Age, Sokoto, Nigeria.

## PREVALENCE DE L'HYDATIDOSE CHEZ LES CHAMEAUX ABATTUS DANS L'ABATTOIR CENTRAL DE SOKOTO AU NIGERIA

### Résumé

Les données sur la prévalence du kyste hydatique chez les chameaux abattus à l'abattoir métropolitain de Sokoto au Nigeria ont été recueillies sur base d'une inspection post mortem sur une période de 2 mois. Des chameaux des deux sexes (mâle et femelle) et de diverses catégories d'âge (pubères, < 3 ans, et post-pubères, ≥ 3 ans) ont été soumis à un examen post mortem. Des 200 chameaux abattus examinés, 84 (42%) étaient infectés. La prévalence de l'infection chez les chameaux âgés de < 3 ans (non encore pubères) était de 39,13%, tandis que chez ceux de ≥ 3 ans (post-pubères) elle était de 42,37%. Des 84 (42%) chameaux infectés, 78 (92,86%) des infections étaient localisées dans les poumons, 21 (25,0%) dans le foie et 10 (11,90%) dans la rate. On n'a noté aucune association statistique entre le sexe et le kyste hydatique, ni entre les différentes catégories d'âge et cette maladie ( $P > 0,05$ ). L'importance zoonotique de cette maladie et les mesures de contrôle proposées ont été abordées.

**Mots-clés:** Hydatidose, abattage, Chameau, Age, Sokoto, Nigeria.

## Introduction

Cystic echinococcosis (CE) is a widespread zoonosis infecting a large number of wild and domestic animals and humans. The agent of this disease is a tapeworm (*Echinococcus granulosus*) from dogs and other canidae whose larval stage develops as a liquid tumor, called a hydatid cyst (Bouree, 2001). Echinococcosis (Hydatidosis) is one of the most important of the forty or so canine-associated zoonoses (Cook, 1989) and among the most geographically widespread of the pathogenic parasitic zoonoses (Schantz, 1991). The infection represents a problem of medical, veterinary, and economic importance in endemic areas (Schantz et al., 1995). CE in farm animals causes considerable economic problems due to loss of the edible liver. Significant loss of meat and milk production and value of the fleece from infected sheep may also occur. These losses are of special significance in countries of low economic output where sheep production is of particular importance (Torgerson et al., 2001).

Six species of *Echinococcus* have been recognized, but four are of public health concern: *Echinococcus granulosus* (which causes cystic echinococcosis), *Echinococcus multilocularis* (which causes alveolar echinococcosis), *Echinococcus vogeli* and *Echinococcus oligarthrus* (which cause polycystic echinococcosis). Two new species have recently been identified: *Echinococcus shiquicus* in small mammals from the Tibetan plateau and *Echinococcus felidis* in African lions, but their zoonotic transmission potential is unknown.

In sub-Saharan Africa, various studies have shown that CE disease is highly endemic (Daniel, 1995; Bouree, 2001; Dalimi et al., 2002). In Sub-Saharan Africa, *E. granulosus* is perpetuated predominantly by domestic cycle involving an array of livestock species which include cattle, camel, sheep, goats, pigs, donkeys and horses (Eugster, 1978). So far, based on the partial sequences of mitochondrial cytochrome oxidase subunit I (COI) and NADH dehydrogenase I (NDI) genes, ten distinct genetic types (G1-G10) of *E. granulosus* have been identified (Bowles and Mc Manus, 1993a; Bowles et al., 1994; Snabel et al., 2000). Recently, *E. granulosus* was divided into the

following groups: *E. granulosus sensu stricto* (G1; sheep strain, G2; Tasmanian sheep strain, G3; buffalo strain), *E. equinus* (G4; horse strain), *E. ortleppi* (G5; cattle strain), *E. canadensis* (G6; camel strain, G7; pig strain, G8; cervid strain, G9; human strain, G10; Fennoscandian cervid strain) (Nakao et al., 2007). Among the genotypes in the *E. granulosus sensu stricto* group, the sheep strain (G1 genotype) has the most wide geographic distribution around the world. The sheep strain is also dominant in the Mediterranean area (Eckert and Thompson, 1997; McManus and Thompson, 2003; Breyer et al., 2004; Romig et al., 2006; Busi et al., 2007; Varcasia et al., 2007). Some of the other genotypes of *E. granulosus* have been reported to occasionally cause infections in human, but some genotypes are not involved in human infections (Bowles and Mc Manus, 1993b; Dinkel et al., 2004).

Infections with *E. granulosus* cysts in intermediate hosts (sheep, goat, cattle, horses, etc.) are typically asymptomatic, except a few cases of long standing and heavy infections, for example in horses. There are no reliable methods for the routine diagnosis of the infection in living animals, but in rare cases cysts have been identified by ultrasonography alone or in conjunction with serum antibody detection (Eckert, et al., 2001). The most reliable diagnostic method is cyst detection during meat inspection or at post-mortem examination.

The population of camel in Nigeria is predominantly in Northern Nigeria particularly in Borno, Kano, Sokoto, Katsina, Kebbi, Jigawa and Yobe states. In semi-arid Northern Nigeria, camels are used as transport animals and the meat of camels is becoming an acceptable food for the public. In Sokoto and Maiduguri camel meat was found to rank second to beef (Mustapha and Oluyisi, 1993; Abubakar and Maigandi, 1994; Agaie et al., 1997).

Relatively high rates of infection have been reported in camels, in part because they are slaughtered at an advanced age, when they no longer have value as transportation. Gusbi et al., (1990) stated that camels were slaughtered in Libya when no longer useful for work, often at an age of 10 to 15 yrs.

## Materials and Methods

### Study Area

The study area was Sokoto Abattoir, Sokoto state, Nigeria. The abattoir supplies the state and other neighboring areas with meat.

Four (4) visits in a week were made to the abattoir in the morning for a period of two (2) months. During these visits, an average number of 11 camels (males and females) were found to be slaughtered in the abattoir on daily basis. Before slaughter, the camels used in the study were marked for proper identification.

### Age of the Camels

The estimated age of each camel was determined using the dentition (the eruption and wearing of the deciduous and permanent teeth). The camel has a reduced dentition. Some teeth are absent thereby resulting into 22 deciduous and 34 permanent teeth (Wilson, 1984). The most convenient teeth for determination of age are the incisors (front teeth), the canines and the first pair of the premolars. The remaining teeth are hidden by the cheeks. In this work, the ages of the camels were estimated after the camels have been slaughtered.

Eruption of central deciduous incisors occurs at birth to 14 days, laterals at 4-5 weeks and corners at 6-12 weeks. At 6 months, some degree of wear may be seen beginning with the centrals. Deciduous canine and upper premolars 1, 2 & 3 and lower premolar 1 & 2 are all obvious by 6 months. The upper temporary corner incisors are the weakest in the whole head and usually disappear by 12 months (Wilson, 1984). At this age (1 year) all the deciduous teeth are fully up and lower incisors are in wear. The first permanent teeth are the first pair of molars at both lower and upper jaws at 12-15 months (Wilson, 1984). By 2 years of age, deciduous incisors show progressive wear and separation; molars 1 start to wear and molars 2 are about to erupt. At 3 years, the incisors are well worn out; and some may be loosening. At 5.5 years, all the molars have erupted.

The incisors are well worn, irregular and loose, and may be reduced to stumps at 4 years. At 4.5-5 years lower deciduous

premolars are shed and are usually not replaced. Permanent central incisors erupt; upper permanent premolars 2 & 3 and permanent lower premolars 2 erupt, deciduous lateral incisors are shed, upper and lower molar 1 & 2 are in wear and molar 3 about to erupt by 5.5 years. At 6-7 years upper corner permanent incisors (not found in every camel), upper and lower permanent canines and upper permanent premolars have all become apparent. Permanent central lateral incisors are in wear and lower permanent corner incisors erupt and develop. At 7 years, premolars and molars are in wear. All permanent teeth are present and incisors wear as at eight years of age. Also premolar 1 when present are darkly stained due to accumulation of tartar (plaque), canines are large and powerful particularly in males.

From 9 years on wards, all teeth are more or less worn but actual age can only be determined on the basis of experience and considerable knowledge. The principal problem in determining the age of camel using teeth alone lies in the fact that all milk teeth are in wear at the age of 4.5-5 years. This can be confused with that of a very old camel. In the young camels, however, the worn out deciduous teeth will be seen as smaller stumps and most if not all, will be loose while the stump will be large and firm in the older camel. In addition, the older camel's gum will be yellowish rather than pinkish. She-camels reach puberty at two years of age but are not usually mated until three years. Male camels are sexually active at three years but are not used as stud animals until five years (Geoffrey *et al.*, 1993).

### Examination for Hydatid Cysts

After slaughter and evisceration, the pluck and the viscera of each identified camel was inspected for presence of hydatid cyst, by visualization and palpation of these organs. The camels diagnosed to have hydatid cyst, and the organ(s) affected were recorded against the sex and age of such identified camels. Identification of the viable metacestodes was carried out by adding a drop of 0.1% aqueous eosin solution to equal volume of protoscoleces on a microscope slide on the principle that viable protoscoleces completely or partially exclude the dye, while

**Table 1:** Prevalence of hydatid cyst disease in camels in relation to sex of camels

Sex	No. of camels examined	No. of camels infected	Percentage (%) infected
Male	97	39	40.21
Female	103	45	43.69
<b>Total and Mean</b>	<b>200</b>	<b>84</b>	<b>42</b>

P=0.6682 (P>0.05)

**Table 2:** Prevalence of hydatid cyst disease in relation to the age of camels

Age category	No. of camels infected	No. of camels infected	Percentage (%) infected
< 3 years (not yet at puberty)	23	9	39.13
≥3 years (beyond puberty)	177	75	42.37
<b>Total and mean</b>	<b>200</b>	<b>84</b>	<b>42.00</b>

P=0.8256 (P>0.05)

the dead ones take it up (Smyth and Barrett, 1980; Macpherson, 1985). Cyst materials were labeled against the species of animals collected from as well as the organ in which it was found. Only metacestodes with viable protoscoleces were recorded and used in this investigation.

**Results**

Results from this investigation revealed that out of overall total of 200 camels examined, 84 (42%) were infected with hydatid cyst disease. Out of a total of 97 male camels, 39 (40.21%) were infected; and out of 103 female camels, 45 (43.69%) were infected (Table 1). Twenty three (23) of the overall total number of camels examined were below 3 years of age (not yet at puberty), out of which 9 (39.13%) were infected; and 177 were above 3 years of age (beyond puberty), out of which 75 (42.37%) were infected (Table 2). There was no statistical association between the sex and the hydatid cyst disease; and between the different age categories and the hydatid cyst disease (P>0.05).

**Discussion**

Out of the two hundred (200) slaughtered camels examined, 84 (42%) were found to be infected. The prevalence rate obtained in this work was, however, lower than the prevalence rate obtained by Garba and Maigandi (1995); and Dada (1980), which were

48.8% and 50.0%, respectively. This may be due to increased awareness of the camel owners over the years on the epidemiology of the disease and/or benefits of routine deworming of these animals and the definitive host of the parasite, the dog.

Camels ≥ 3 years of age (beyond the age of puberty) had an infection rate of 42.37%, while those of < 3 years of age (not yet at puberty) had 39.13%. Although infection might have occurred at youthful age of the camel, development of the cyst up to the size that can easily be identified by palpation on post-mortem examination will require some years. Hydatid cysts grow slowly at rate of about 1 cm/year (Zakim and Boyer, 1982). This, could explain the reason for the lower prevalence obtained in younger camels in this study. It has also been shown that there is a high correlation between the infection and the age of camels (Soulsby, 1982 and Shambesh, 1997).

Out of the 84 (42%) camels infected, 78 (92.86%) of the infections were in the lungs; 21 (25.0%) in the liver; and 10 (11.90%) in the spleen. Single organ infection with hydatid cyst in all the camels studied was observed only in the lungs. As reported by Dada *et al.*, (1980), when cysts were observed in the liver, spleen, or other organs, they were also present in the lungs.

The prevalence of hydatidosis in camel revealed by this work highlights the potential danger which infected camels may constitute to dogs and subsequently to humans and other

animals intermediate hosts. At least five out of ten *E. granulosus* strains (designated G1 – G10) are infective to humans in sub Saharan Africa. Most cases of CE are caused by the sheep strain (G1) and camel strain (G6). It is therefore, recommended in Nigeria, especially northwestern part, camel hydatid cyst disease should be taken more seriously since there is an increase in the consumption of camel meat in addition to the various uses that camels are being put to in Sokoto in particular and Nigeria in general such as their use as drought animals; and for milk due to its rich nutritional composition. Other strains occurring in the area are horse strain (G4 or *E. equines*), and cattle strain (G5 or *E. orteleggi*) and probably a lion strain (Macpherson and Wachira, 1997; Dinkel *et al.*, 2004). Implementation of proper meat hygiene and inspection should also be given due priority among other approaches.

## Impact

Camels are becoming increasingly important source of protein in different regions of the world, especially where the animal is bred in abundance. At the same time, it can also serve as a source of hydatid cyst disease through the dog, the definitive host of the helminth parasite, *E. granulosus*. This is in addition to production losses occasioned by the disease burden. It is therefore important that these animals are routinely treated against such worms and dogs prevented from consuming the cysts of the parasite removed from the carcass during meat inspection.

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## EFFECTS OF REPLACING FISHMEAL WITH SOYABEAN IN DIETS OF OREOCHROMIS NILOTICUS FINGERLINGS IN BUEA, CAMEROON

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### Abstract

Oreochromis niloticus fingerlings were fed four with isoproteic (30% crude protein) diets in which soya bean meal (SBM) partially or completely replaced fishmeal (FM), and the effect of the diets on the growth performance and nutrient utilization of the fingerlings was evaluated. 80 fingerlings of mean weight 5.594g were distributed among 8 transparent plastic tanks (10/tank) with two replicates per treatment and the trial lasted 56 days. The control (Diet I) had no replacement of FM with SBM while the experimental diets: Diet II, Diet III and Diet IV had 35%, 65% and 100% replacement respectively. There were significant differences in the mean total weight gain at  $P < 0.05$ , with fingerlings fed Diet III having the greatest mean total weight gain ( $3.695 \pm 0.061$  g) and those in Diet II, the least ( $0.930 \pm 0.388$ g). The total percentage weight gain and the apparent protein efficiency ratio followed the same trend. Diet III was the most palatable diet with the greatest mean final feed intake value ( $1.853 \pm 0.004$ g). Percentage digestibility and feed conversion efficiency values of diets also increased with increasing replacement of FM. The effective substitution of 65% fishmeal by soya bean without negative effects on fish condition has positive implications for increased fish production in Cameroon from aquaculture.

**Key Words:** Fishmeal, Soyabean meal, Diets, Oreochromis niloticus, Cameroon.

## EFFETS DU REMPLACEMENT DE LA FARINE DE POISSON PAR LA FARINE DE SOJA DANS LES REGIMES DES ALEVINS Oreochromis niloticus A BUEA (CAMEROON)

### Résumé

Des alevins Oreochromis niloticus ont été nourris sur base de quatre régimes isoprotéiques (30% de protéine brute) dans lesquels la farine de soja (FS) a partiellement ou totalement remplacé la farine de poisson (FP) ; et l'effet de ces régimes sur la croissance et l'utilisation des éléments nutritifs par les alevins a été évalué. Quarante-vingt (80) alevins d'un poids moyen de 5,594g ont été répartis dans 8 cuves en plastique transparentes (10/cuve) avec deux répétitions par traitement, et l'essai a duré 56 jours. Dans le régime témoin (régime I), il n'a pas eu de remplacement de farine de poisson par la farine de soja, tandis que les régimes expérimentaux - régimes II, III et IV ont respectivement eu des remplacements de 35%, 65% et 100%. On a noté des différences significatives au niveau du gain pondéral total moyen à  $P < 0,05$ , les alevins nourris au régime III ayant le plus grand gain pondéral total moyen ( $3,695 \pm 0,061$  g) et ceux du régime II ayant le plus faible gain pondéral total moyen ( $0,930 \pm 0.388$ g). Le pourcentage total du gain pondéral et le coefficient d'efficacité protéique apparente ont suivi la même tendance. Le régime III était le plus agréable au goût, avec la plus grande valeur moyenne d'absorption alimentaire finale ( $1,853 \pm 0.004$ g). Les valeurs de pourcentage, de digestibilité et d'efficacité de conversion des aliments des régimes ont également augmenté parallèlement au remplacement croissant de la farine de poisson. La substitution efficace de 65% de la farine de poisson par la farine de soja, sans effets négatifs sur l'état des poissons, a des implications positives pour l'augmentation de la production de poissons au Cameroun.

**Mots-clés:** Farine de poisson ; Farine de soja ; Régimes ; Oreochromis niloticus ; Cameroun.

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## Introduction

The need for dietary protein which is important for growth and good health continues to increase as the world population grows. The pressure is felt in the world as a whole and the developing countries in particular. Therefore, increasing protein supply is imperative. When compared with other animal proteins such as cattle, pig, poultry which are expensive due to low technological level and poor pasture lands, fish provides throughout the tropics a cheaper source of first class proteins for human consumption. For this reason, most countries in the tropics, including Cameroon, have turned their attention, more than ever before, to the development and exploitation of their fisheries resources and production of fish by culture as a means of providing their citizens with the much needed animal proteins (Moses, 1983; Odedeyi, 2007). *Oreochromis niloticus* (Nile Tilapia) is one of the most cultured species in the country due to its high resistance to disease, tolerance to a wide range of environmental factors, fast growth rate and fast adaptability to artificial diets (Ugwumba, 1988, as cited by Ogunji and Wirth, 2000). There exist a few or no cultural, religious or social prohibitions to its consumption, (for example some Cameroonian tribes forbid scaleless fish like the catfishes) which has led to its wide acceptance by populations of diverse cultural beliefs. It is therefore considered one of the fish species with great potential for culture in Cameroon.

Fish feed amounts to over two thirds or 60 - 70% of the running cost of culturing fish in an intensive management system (Eyo, 1990 cited in Eyo, 1994). Fish should therefore be fed at quantities and qualities which do not exceed their dietary requirements in order to minimize wastage (Fafioye *et al.*, 2005). The lack of adequate fish feed is one of the most critical problems faced by the aquaculture industry in Cameroon (Folack *et al.*, 2000). This is due, partly, to the lack of technical knowledge by operators on the use of potential, readily available and cheap protein sources from which the feed could be formulated. The result of this is overdependence on expensive importation of supplemental protein sources such as fish meal,

thus rendering fish feeds expensive. Enzenwa (1979) and Agbebi *et al.*, (2009) observed that the cost of feeds became a disincentive to small scale fish farming in Nigeria since interested small holders could not afford the expensive yet inevitable feed for their stocks.

Based on cost effectiveness, availability, crude protein content, amino acid profile, and energy content, several feed ingredients have been investigated in an attempt to substitute the traditional fish meal as a protein source. These include animal and plant protein sources (Ogunji and Wirth, 2000, 2001). Such fish nutrition studies are however scanty in Cameroon. This study carried out at the foot of Mt. Cameroon in Buea, therefore sought to evaluate the possibility of totally or partially replacing the expensive and conventional fishmeal in *O. niloticus* diets with the cheaper and readily available soya bean meal to reduce the cost of fish feeds and consequently making fish culture a less expensive and more economical venture in the country. The choice of soya bean was further enhanced by the fact that soya bean production is undertaken in many areas of Cameroon and its transformation and processing practised on large scale by Cameroonian companies such as TANTY Ltd. which is specialized in the growth, transformation and distribution of soya bean products (Azabji *et al.*, 2011)

## Materials and Methods

Four isoproteic diets (30% crude protein) were formulated as shown in Table I. The control (Diet I) had a 0% replacement of FM with SBM, and the experimental diets: Diet II, Diet III and Diet IV had 35%, 65% and 100% replacement of FM with SBM, respectively. Each diet differed from the other mainly by the percentage of replacement of fish meal with Soya bean meal, the two major dietary protein sources in this study. To maintain the isoproteic nature of formulated diets, adjustments were made in the inclusion of other major nutrients particularly the carbohydrate sources (corn flour and wheat bran) as soya bean meal was progressively replaced by fish meal in the experiment. All other nutrients which could not substantially affect protein levels were

maintained at constant levels in all the diets. This was also the case in previous studies carried out by Gaber (2006) and Rawles *et al.*, (2011).

Locally procured feed ingredients of which the types and amounts are shown in Table I, bought from a livestock shop in Bonaberi, Douala in Cameroon were individually ground using a locally made two-hammered grinder to a fine particulate size of about 0.1mm to enable pelleting. They were then weighed out precisely for the respective diets (Table I) and thoroughly mixed manually using a spade several times to ensure homogeneity, after which varying quantities of warm water were added to each diet. This was then felt manually until a convenient paste suitable for pelleting was formed. The paste was completely homogenized and extruded through a 1.5mm pore size of the pelleting compartment of the same machine that was used for grinding. There was difficulty in pelleting diet IV which was attributed to the relatively high content of soya bean which conferred a high fibre content to it and low content of corn which also served as a binding ingredient. After pelleting, diets were thoroughly sun-dried and checked intermittently until moisture could no longer be hand-felt, packaged into air-tight locally produced unbranded medium size polythene plastic bags and stored in cold dry cupboards to prevent moulding or rancidity. Proximate analysis of diets was conducted according to methods outlined by Association of Official Analytical Chemists (AOAC, 1995) (Table I).

Fingerlings of the Nile Tilapia were harvested from the Great Soppo Hatchery Complex, Buea and transported immediately to the laboratory in a 100-litres container with water at 23°C from the hatchery tanks. In the laboratory, 80 of these fingerlings were randomly selected by collecting from different areas with a hand net, weighed using a top-loading electronic balance (mean individual weight: 5.594g) and stocked in groups of ten, into eight 23-litres cylindrical transparent experimental plastic tanks where they were acclimatized for a period of one week. During acclimatization, fingerlings were fed a common diet formulated in the Fisheries and Oceanography Research Centre, Batoke, Limbe

(Table I). Experimental tanks were covered with mosquito nets to prevent entrance of insects and other organisms that could pollute water and affect feed composition. Each of the four treatments was replicated twice and the study lasted for 56 days (8 weeks) as previously recommended by FAO (1986). Fish were manually hand-fed with their respective diets twice daily between 8:00 - 9:00 and 16:00 - 17:00, at a rate of 3% of fish biomass in each tank. Left-over food was routinely removed by siphoning before feeding. At the end of each week the fish were measured and weighed. The amount of feed fed was adjusted each week to accommodate the new weight attained. The experimental water used in the aquaria was dechlorinated municipal tap water which was achieved by exposing the fetched tap water to air in a large mouthed 200-litres container for a period of about 48 hours to enable the evaporation of chlorine gas. Partial exchange of water was done by siphoning daily to remove faeces and leftover food while complete water exchange and cleaning of aquaria was done once a week to ensure good water quality. Physical and chemical parameters of the water were taken. Temperature was measured twice daily, while pH, dissolved oxygen concentration and ammonia were taken weekly. The water quality parameters are presented in Table 3.

At the end of the trial period, growth indices and nutrient utilization parameters were calculated for each treatment. The indices were calculated as described in Jamabo and Alfred-Ockiya (2008) and Fafioye *et al.*, (2005). The arithmetic formulae used to calculate the indices/ parameters were as follows:

#### Growth Performance Parameters

- Mean weight gain (g) =  $W2 - W1$     Where  
 $W1$  = Initial mean weight (g);  $W2$  = Final mean weight (g)
- Total percentage weight gain (%) =  $\frac{\text{Total weight gain}}{\text{Initial weight}} \times 100$
- Survival Rate (%) =  $\frac{nf \times 100}{ni}$     Where  
 $nf$  = Number of surviving fish;  $ni$  = Number of fish from start of experiment

#### Feed Utilization Parameters

- TEFI (Total Effective Feed Intake) =  $\frac{\text{Total Feed dispensed} - \text{Total Feed leftovers}}{\text{Total weight gain}}$

siphoned.

- Mean Daily Feed Intake (g/day) = Mean total feed intake (g) / T Where T = Time in days
- Mean Feed Intake per Week = Mean total feed intake (g) / n Where n = Number of weeks of the experiment
- % Digestibility =  $\frac{D_i - D_f}{D_i} \times 100$  Where  $D_i$  = Dry weight of feed intake;  $D_f$  = Dry weight of faeces
- Feed Conversion Ratio (FCR) = Feed intake/Live weight gain
- Protein intake (PI) = Protein content x Mean Daily Feed Intake
- Protein efficiency ratio (PER) = Fish weight gain (g) / Protein intake (g)

Results were subjected to a non-parametric Kruskal Wallis test using the SPSS Standard Version, Release 12.0 (SPSS Inc. 1989-2003) statistical software. Comparisons between means were performed using the Wilcoxon U-test and Mann-Whitney U-test.

## Results

Dissolved oxygen values throughout the experimental period ranged from 4mg/l to 6mg/l, with diet II having the greatest mean dissolved oxygen value (5.7 mg/l) and diets III and IV having the least (4.9 mg/l). pH values ranged from 7.5 to 8.5 with mean pH values being equal (7.7) for all treatments. Ammonia values were constant throughout the experimental period for all treatments. Temperature of experimental water ranged from 23°C to 26°C.

The ingredients and proximate composition of the diets are presented in Table 1. Dietary crude lipids (fats) levels ranged from 5.34% to 8.18%. Crude fibre levels increased from diet I to diet IV with increasing inclusion of soya bean meal ranging from 6.40% to 9.94%. Carbohydrates expressed as % of nitrogen free extracts (NFE) ranged from 32.18% to 40.75% with that of diet III being slightly lower than diet II. Growth responses of fingerlings to diets and nutrient utilization parameters are summarized in Table 2. Mean total weight gain values were significantly different ( $P < 0.05$ ) between treatments. Diet III (65% replacement of FM

with SBM) was observed to have the greatest weight gain ( $3.70 \pm 0.061$ g) while Diet II had the least ( $0.93 \pm 0.388$ g). By the end of the trial period, the greatest final weight was in Diet III ( $9.22 \pm 0.186$  g) and the least was observed in Diet I ( $5.81 \pm 0.255$  g). Diets III and IV had the highest survival rate (100% survival), while Diet II had the least survival rate (highest mortality) (70% survival or 30% mortality). One fish died in Diet I, while six died in one replicate of Diet II with all surviving in the other replicate.

Various levels of the estimated consumed food (feed intake) are shown in Table 2. There was a significant difference in the mean final feed intake between all treatments ( $P < 0.05$ ) (Table 2). At the start of the experiment, the greatest amount of feed intake was observed in Diet IV while the least was observed in Diet I. By the end of the trial, however, the greatest amount of feed intake was observed in Diet III while the least was maintained in Diet I. The feed conversion efficiency increased in the following order: Diet I, II, IV and III with Diet III having the highest feed conversion efficiency ( $27.53 \pm 0.386$ ) and Diet I the least ( $14.49 \pm 0.404$ ). Protein intake values increased from Diet I (2.526) to Diet IV (4.340) and the apparent protein efficiency ratio had values ranging from 0.277 in Diet II to 0.906 in Diet III. Percentage digestibility of diets ranged from 77.570 in Diet I to 85.129 in Diet III (Table 2). It was observed that percentage digestibility increased with increasing substitution with soya bean meal until it reached the 65% replacement level (Diet III), after which digestibility reduced.

Results of the economic evaluation of the diets are presented in Tables 4 and 5. From the evaluation of the formulation and compounding cost of 100 kg of each of the experimental diets, it was noticed that Diet III was the cheapest to formulate and compound (27,995 FCFA/100kg bag) Diet I was the most expensive (29,487 FCFA/100kg bag).

## Discussion

From the results obtained in this study, soya bean meal can suitably substitute up to 65% of fish meal in the diets of *Oreochromis niloticus* fingerlings without any negative effect on fish's condition. It is also evident

**Table 1:** Percentage composition of diets formulated using partial or complete replacement of Fish meal with Soya bean meal

Ingredients	Diet I (0%)	Diet II (35%)	Diet III (65%)	Diet IV (100%)
Corn flour	30.92	(35%)	Diet III	19.16
Wheat bran	30.92	(65%)	Diet IV	19.16
Fish meal	29.16	(100%)	10.21	0.00
Soya bean meal	0.00	10.62	27.95	52.68
Palm oil	5.00	5.00	5.00	5.00
Vitamin/mineral premix	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25
Bone meal	0.25	0.25	0.25	0.25
Oyster shell	0.25	0.25	0.25	0.25
White tapioca	1.25	1.25	1.25	1.25
<b>Proximate composition</b>				
Moisture (%)	8.97	11.17	7.48	6.56
Ash (%)	15.85	10.74	10.59	6.57
Crude Protein (%)	29.48	28.62	30.40	30.84
Crude Lipid (%)	7.12	5.47	8.18	5.34
Crude fibre (%)	6.40	7.99	8.28	9.94
Nitrogen Free Extract (NFE) (%)	32.18	36.01	35.07	40.75

that *Oreochromis niloticus* fingerlings can better utilize diets having an appropriate mixture of SBM and FM as protein supplements than those having entirely FM or SBM since Diet III performed better than Diet I and Diet IV which had entirely FM and SBM as protein supplements respectively.

Generally, the recorded compositions of the diets were within the recommended range for good growth of *O. niloticus* as reported by earlier researchers (Balarin and Hatton, 1979 and Ajani, et al., 2004). The dietary crude protein level of experimental diets which ranged from 28.62% to 30.84% was within the 28% -35% range of crude protein requirement recommended for *O. niloticus* fingerlings (Luquet and Moreau, 1989). The dietary crude lipids (fats) levels ranging from 5.34% to 8.18% obtained in this research could be tolerated by *O. niloticus* which is not a high lipid requiring fish unlike salmonids which need a lipid level as high as 12% (Stickney and Hardy, 1989). Crude fibre values ranged from 6.40% to 9.94% which corresponded to the optimal crude fibre range for Tilapia (6-10%) as observed by Anderson et al., (1984) cited by Shiau et al., (1988). Shiau et

al., (1988) reported that higher than required fibre content decreased utilization of feed by hastening gastric emptying time in Tilapia. The present experiment corroborates this observation since fish fed Diet IV (which had relatively higher crude protein) experienced the highest faeces output and did not attain the highest weight gain despite the fact that Diet IV also had the greatest crude protein level and experienced the highest consumption or feed intake level. Diet III had a better weight gain implying that it was better utilized and had probably attained the optimum value of soyabeans replacement required for the fish. Mean weight gain also depended on digestibility of diets. Diet III was observed to have the greatest digestibility evidenced by the fact that the least amount of feed left over was siphoned from the tanks in which Diet III was fed. These results agree with the findings of Stickney and Hardy (1989) who observed that some chemical and physical properties of the diet as they relate to fibre content, flavour, texture and colour could affect acceptability. The increase in weight gain of fish with a corresponding increase in level of crude protein supported the

**Table 2:** Growth Performance and Feed Utilization Parameters of *O. niloticus* fingerlings

Parameters	Diet I Control	Diet II 35%	Diet III 65%	Diet IV 100%	Kruskal Wallis Test
Mean Initial wgt (g)	4.570±0.287 <sup>abc</sup>	5.895±0.098 <sup>ad</sup>	5.525±0.247 <sup>be</sup>	6.385±0.054 <sup>cde</sup>	P< 0.001
Mean Final wgt (g)	5.810±0.255 <sup>abc</sup>	6.825±0.290 <sup>ade</sup>	9.220±0.186 <sup>bd</sup>	9.095±0.063 <sup>ce</sup>	P< 0.001
Mean Total wgt gain (g)	1.240±0.032 <sup>ab</sup>	0.930±0.388 <sup>cd</sup>	3.695±0.061 <sup>ace</sup>	2.710±0.009 <sup>bde</sup>	P< 0.001
Mean Weekly wgt gain (g)	0.155±0.004 <sup>ab</sup>	0.116±0.048 <sup>cd</sup>	0.462±0.008 <sup>ace</sup>	0.339±0.001 <sup>bde</sup>	P< 0.001
Mean DGR (g/day)	0.022±0.001 <sup>ab</sup>	0.017±0.007 <sup>cd</sup>	0.066±0.001 <sup>ace</sup>	0.048±0.000 <sup>bde</sup>	P< 0.001
Specific Growth Rate (%)	0.465±0.036 <sup>ab</sup>	0.235±0.106 <sup>c</sup>	0.942±0.045 <sup>acd</sup>	0.632±0.003 <sup>bd</sup>	P< 0.001
Total % Wgt Gain	30.233±2.600 <sup>ab</sup>	17.936±6.874 <sup>cd</sup>	70.479±4.246 <sup>ace</sup>	42.478±0.215 <sup>bde</sup>	P< 0.001
Survival Rate (%)	95.00	70.00	100.00	100.00	P< 0.001
Mean initial feed intake (g)	0.825±0.003 <sup>abc</sup>	1.086±0.003 <sup>ad</sup>	1.112±0.006 <sup>be</sup>	1.239±0.003 <sup>cde</sup>	P< 0.001
Mean final feed intake (g)	1.056±0.008 <sup>abc</sup>	1.409±0.001 <sup>d</sup>	1.853±0.004 <sup>abde</sup>	1.801±0.006 <sup>ce</sup>	P< 0.001
Mean Total feed intake (g)	8.568±0.017 <sup>abc</sup>	11.718±0.006 <sup>ade</sup>	13.411±0.033 <sup>bdf</sup>	14.073±0.034 <sup>cef</sup>	P< 0.001
Mean Weekly feed intake (g)	1.071±0.002 <sup>abc</sup>	1.465±0.001 <sup>ade</sup>	1.676±0.004 <sup>bdf</sup>	1.759±0.004 <sup>cef</sup>	P< 0.001
Mean daily feed intake (g)	0.153±0.000 <sup>abc</sup>	0.209±0.000 <sup>ade</sup>	0.239±0.001 <sup>bdf</sup>	0.251±0.001 <sup>cef</sup>	P< 0.001
Mean total Faeces dry wgt (g)	1.922±0.009 <sup>abc</sup>	2.268±0.055 <sup>ade</sup>	1.995±0.02 <sup>bdf</sup>	2.481±0.034 <sup>cef</sup>	P< 0.001
FCR (g)	7.006±0.195 <sup>abc</sup>	5.493±2.284 <sup>ad</sup>	3.645±0.051 <sup>be</sup>	5.195±0.030 <sup>cde</sup>	P< 0.001
FC Efficiency (%)	14.488±0.404 <sup>ab</sup>	17.966±3.312 <sup>c</sup>	27.534±0.386 <sup>ad</sup>	19.262±0.112 <sup>bcd</sup>	P< 0.001
% Digestibility of Diet	77.570±0.060 <sup>abc</sup>	80.645±0.464 <sup>ade</sup>	85.129±0.113 <sup>bdf</sup>	82.380±0.200 <sup>cef</sup>	P< 0.001
Apparent Protein Intake (g)	2.526	3.354	4.077	4.340	P< 0.001
Apparent Protein Efficiency	0.491	0.277	0.906	0.624	P< 0.001
Nitrogen Metabolism	18.749±0.486 <sup>ab</sup>	14.062±5.862 <sup>cd</sup>	55.868±0.919 <sup>ace</sup>	40.975±0.139 <sup>bde</sup>	P< 0.001

\*Means with the same superscript in the same row are not significantly different P>0.05.

findings of Akand *et al.*, (1989) who observed a similar increase in mean weight gain when utilizing increasing propotion of dietary protein levels from 19.87% to 35.43%. The observed survival rate seemed not to have been due to any particular attribute of the diets. This is because the high mortality observed in diet II was experienced only in one replicate. This was probably due to fighting resulting from competition for food and space and size domination since the surviving fish in this replicate were all larger than those that died but no firm conclusions could be taken, since

no tests were made in this study to investigate the possible causes. The high final feed intake of Diet III indicated that it was the most palatable diet. Palatability is positively correlated to feed intake.

Diet III which had the greatest digestibility percentage (85.129%) was most properly digested and assimilated with very little wasted as faeces, while Diet I was the least digested. This could be due to Diet III having an appropriate balance of ingredients, evidenced by its relatively higher acceptable crude lipid (8.18%), appropriate level of crude protein

Treatments	Statistics	Dissolved oxygen (mg/l)	pH	Ammonia (mg/l)	Temperature (°C)
Treatment 1	Mean±S.E	5.1±0.3	7.7±0.1	3.0±0.0	24.4±0.3
	Range	4-6.0	7.5-8.0	3.0-3.0	23-26.0
	Std. Deviation	0.8	0.3	0.0	1.0
Treatment 2	Mean±S.E	5.7±0.2 <sup>ab</sup>	7.7±0.1	3.0±0.0	24.0±0.2
	Range	5.0-6.0	7.5-8.0	3.0-3.0	23.0-25.0
	Std. Deviation	0.5	0.3	0.0	0.7
Treatment 3	Mean±S.E	4.9±0.3 <sup>a</sup>	7.7±0.1	3.0±0.0	24.4±0.3
	Range	4.0-6.0	7.5-8.5	3.0-3.0	23.0-26.0
	Std. Deviation	0.8	0.4	0.0	0.9
Treatment 4	Mean±S.E	4.9±0.2 <sup>b</sup>	7.7±0.1	3.0±0.0	24.1±0.2
	Range	4.0-6.0	7.5-8.0	3.0-3.0	23.0-25.0
	Std. Deviation	0.6	0.3	0.0	0.6
Kruskal Wallis Test (significant level)		P= 0.068	P= 0.959	P=1.000	P= 0.640

\*Means with the same superscript are not significantly different at  $P>0.05$ .

**Table 4:** Quantity of individual ingredients required to formulate 100kg experimental diet

Ingredients	Diets and Quantity of ingredient (Kg/100Kg feed )			
	Diet I	Diet II	Diet III	Diet IV
Corn flour	30.92	30.72	26.42	19.16
Wheat bran	30.92	30.72	26.42	19.16
Fish meal	29.16	18.95	10.21	0.00
Soya bean meal	0.00	10.62	27.95	52.68
Palm oil	5.00	5.00	5.00	5.00
Vitamin/mineral premix	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25
Bone meal	0.25	0.25	0.25	0.25
Oyster shell	0.25	0.25	0.25	0.25
White tapioca (garri)	1.25	1.25	1.25	1.25

(30.40%), and a fibre content that allows for appropriate digestion in fish (8.28%). The low digestibility of Diet I could be attributed to its relatively low crude fibre content that was not enough to allow for the feed to move in bulk in the gastrointestinal tract. This supports the observations made by Shiau *et al.*, (1988) that lower than normal fibre in diets could limit nutrient digestion.

The protein efficiency ratio was, however, not consistent with protein intake. The trend showed by protein efficiency (Diet II, Diet I, Diet IV, and Diet III) explained the fact

that Diet III had the most utilized protein than all the other diets, and that Diet I (control) could be promising if not limited by its low digestibility resulting from low fibre content and also by its relatively low crude protein percentage. The trend of protein efficiency is similar to that of total percentage weight gain. The protein efficiency ratio values however were all below the range of 1.28 to 2.96 reported by Jauncey and Ross (1982) for *Sarotherodon mossambicus*.

Generally, the physico-chemical parameters of experimental water between

**Table 5:** Cost of individual feed items used and total price of each experimental diet (CFA)

Ingredients	Cost of item* (CFA#/kg)	Cost of food item (CFA) 100 kg-I bag of feed			
		Diet I	Diet II	Diet III	Diet IV
Corn	200	6,184	6,144	5,284	3,832
Wheat bran	100	3,092	3,072	2,642	1,916
Fishmeal	450	13,122	8,528	4,595	00
Soya bean meal	300	00	3,186	8,385	15,804
Palm oil	1000	5,000	5,000	5,000	5,000
Vitamin/ mineral premix	650	1,300	1,300	1,300	1,300
Salt	150	38	38	38	38
Bone meal	250	63	63	63	63
Oyster shell	250	63	63	63	63
White tapioca	500	625	625	625	625
<b>Total cost</b>		<b>29,487</b>	<b>28,019</b>	<b>27,995</b>	<b>28,641</b>

\*The cost of each food item (CFA) in a particular diet was calculated by multiplying the price per kg by the quantity required for that diet (kg).

#\$1US is approximately equal to CFA 500.

treatments were not significantly different in most cases. Results obtained were similar to those reported by Fafioye et al., (2005) who observed that the composition of diets formulated with differentially treated soyabean as main protein source had no effect on the physico-chemical parameters of experimental water and hence no effect on fed *Clarias gariepinus* fingerlings.

The cost-benefit evaluation of the study supports the assertion that using a cheaper and locally available protein supplement such as soya bean to replace fish meal could significantly reduce production cost in fish farming in Cameroon. Considering that high feed cost reduces the profitability of an aquaculture enterprise, Diet III (containing 65% replacement of fishmeal with soya bean meal) from the study was considered to be the most profitable. The replacement of fishmeal with soyabean meal at 65% for *O. niloticus* fingerlings is therefore recommended for fish farmers in Cameroon and other developing African countries in order to establish less expensive, yet more profitable aquaculture ventures.

**Impact**

The study revealed that Soya bean

meal (SBM) can effectively replace up to 65% of Fish meal (FM) in the diets of Nile Tilapia (*Oreochromis niloticus*) fingerlings. It also showed that *O. niloticus* fingerlings makes better use of diets containing a mixture of both plant (SBM) and animal (FM) protein sources rather than of animal protein(FM) only. The best performing diet (65% SBM) was also the cheapest to formulate. It is therefore recommended that efforts be made to educate and encourage fish farmers in Cameroon and other African countries to use the replacement ratio of 65%SBM: 35%FM in their fish diets. This could maximize profit in Nile Tilapia farming whilst also reducing its cost in the local market

**Acknowledgements**

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## SHORT COMMUNICATION

### SURVEY OF THE ABNORMALITIES OF THE GENITALIA OF SLAUGHTERED COWS IN LAFENWA ABATTOIR, ABEOKUTA

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Food and Agriculture of the United Nations Organization, FAO (1989) observed that drought and natural disasters have caused a reduction in protein supply from plant sources. This is highly evident in famine-ridden and war-torn countries generally and most especially in the developing nations, where rural-urban migration and other socio-economic factors have greatly affected agricultural productivity. The surge in human population therefore, would only subsist on increased supply of animal protein, which can be effected through improved productivity in the livestock industry (Olurode, 2002).

The Federal Department of Livestock and Pest Control Services, FDLPCS (1984), estimated the livestock population in Nigeria to be about 13million cattle; 8.5 million sheep and 21 million goats.

Despite these statistics, the daily animal protein intake per head is still very low compared with the average standard recommended by the FAO of the United Nations. The average daily animal protein intake per head in Nigeria is 9.0g (Larmode, 1985) compared with 35.0g recommended by the World Health Organization (WHO). The daily animal protein intake per head was reported as 10.8g for Africa, 41.6g for Europe and 69.0g for North America whilst the world's average is 24.0g (FAO, 1989). Thus, Africa's protein intake is less than 50% of the world's average.

The major sources of animal protein are livestock species like cattle, sheep, goats, poultry (meat and egg), fish and wildlife, etc. In order to meet the FAO recommended average of daily animal protein intake, there should be a deliberate effort at increasing the present population of our livestock especially those

that are locally available that can withstand the environmental stress of the region (Olurode, 2002).

Genital abnormalities play an important role in animal breeding either by causing infertility or sterility, and thus inflict heavy economic losses to the livestock owners. Many cows with reproductive problems and low milk production have been sold or sent to slaughterhouses (Abubakar, 2007). For minimisation of these losses, it is important that disorders of genital organs and their incidence be defined which would serve as baseline data for subsequent research in the study area.

## Materials and Methods

### Study area

The Lafenwa municipal abattoir is in the Abeokuta North Local Government Area of Ogun State. Ogun State is located on geographical map reference of Latitude 3°19.665'E and Longitude 7°09.775'N and at an elevation of 169 feet with an area of 16,762km<sup>2</sup> and a population of 4,054,272 (Takeet *al.*, 2009). The abattoir supplies a large percentage of meat consumed by the population in and around Abeokuta.

Sample Collection time was between 0700 and 0900 hours. Two hundred reproductive tracts of cows were collected at random between May 2009 and July 2009 from Lafenwa abattoir, Abeokuta, Ogun State. No information regarding the identity and history of the animals was included in this study.

The genital organs were transported in cellophane bags to the Theriogenology laboratory at the College of Veterinary Medicine, University of Agriculture, Abeokuta,

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for examination within two hours of collection.

## Method of Examination

### Gross Examination Genital Samples

During gross examination, the genital organs were thoroughly examined visually and manually for the presence of various pathological abnormalities such as colour, consistency, shape, size, cysts, tumours and also examined for the presence of foetus. Vulva was examined for tick infestation. The ticks were identified as described by Hendrix and Robinson (2006). Lesions were diagnosed, evaluated by measurement using ruler and calliper and then photographed.

The vagina and uterus were opened up to utero-tubal junction and examined. The patency of each oviduct was checked by injecting a colour fluid (Indian ink) near the junction of the uterine tube with the corresponding uterine horn. Free flow of the fluid observed was indicative of non-obstruction of the oviducts. Ovaries were inspected for gross lesions.

### Histopathology

Representative samples of some pathological conditions were dissected and fixed in 10% buffered Formalin solution, processed by the paraffin method. Sections were cut at 5  $\mu$ m and stained with haematoxylin and eosin (H&E) for histological examination.

## Results

The results of survey of the abnormalities of female reproductive tracts of cattle from Lafenwa abattoir slaughter slab in Abeokuta are presented in Table 1 while Table 2 shows the state of the uteri encountered. A total of two hundred samples were collected and all had one form of the abnormalities or the other.

The study indicated that 38.5% of the animal sample collected were pregnant, while the result of the gross examination revealed that 50.0% of the samples had vulvae tick infestation, 28.5% endometritis, 14.5% ovarian tumour and 7.0% with ovarian hypoplasia.

Most of the pregnant cows had normal

body condition. Inactive cows presented small smooth ovaries with no follicular development. Left horn was the pregnant horn and no case of twinning found.

Ticks found in the vulva were identified to belong to the following genera: *Amblyomma*, *Boophilus* and *Rhipicephalus*.

Histopathology of the uterine samples showed diffuse mononuclear cells, degeneration of the endometrial glands, presence of plasma cells and macrophages with haemosiderosis and desquamation of the epithelium.

## Discussion

The data obtained from this study reflects high incidence of gross abnormalities of the genitalia of cows slaughtered at Lafenwa abattoir which is highly significant. The lesions were consistent with what was earlier described by Craig (1979).

Most of the lesions were acquired as manifested by the high incidence of endometritis which could have probably been due to contamination at breeding and post partum bacterial complications.

Factors such as twinning, dystocia, retained placenta, metabolic disorders and age of cow played a role in post partum uterine infections (Studer and Morrow, 1978; Roberts, 1986).

Small smooth ovaries devoid of dominant structures characterised by the high incidence of ovarian inactivity was noted in this study. This can be attributed to malnutrition and not to hypoplasia of hereditary origin (Farin and Estill, 1993).

Nutritional deficiency and energy diets were the major causes of inactive ovaries observed in cows during post partum period (Butler and Smith, 1989) and in anoestrous pre-service cows (McDougall *et al.*, 1995). No ovaribursal adhesion was encountered in this present study.

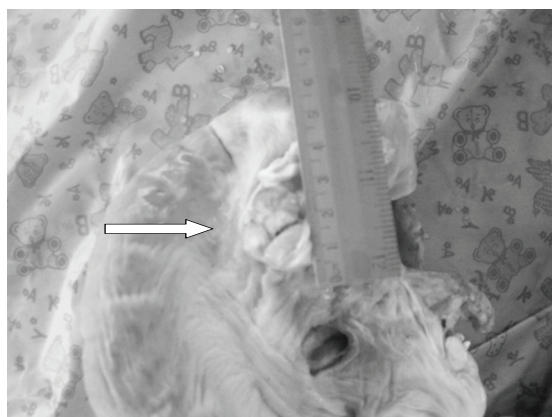
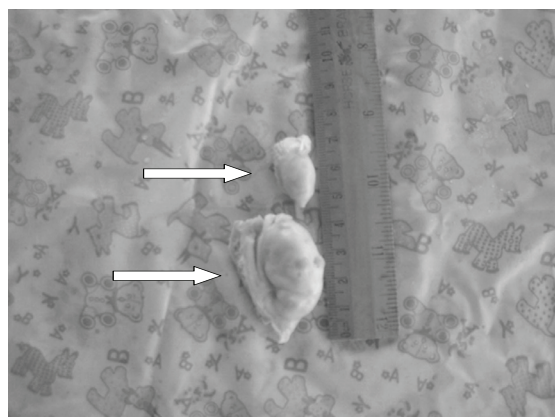
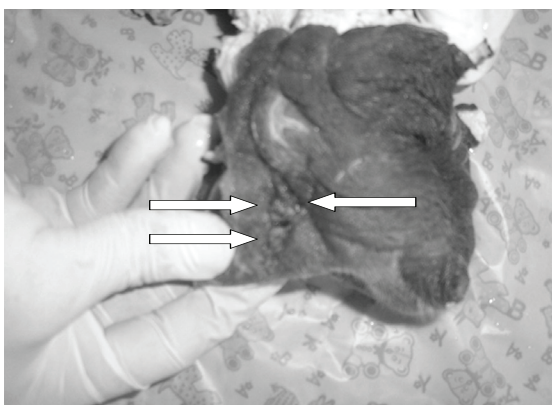
The high incidence of vulvae tick infestation observed in this study correlate well with those obtained by Ribadu *et al.*, (1998). Probable causes of these infestations as recorded in this work are environment and husbandry system. The high incidence recorded in this study is directly related to the period

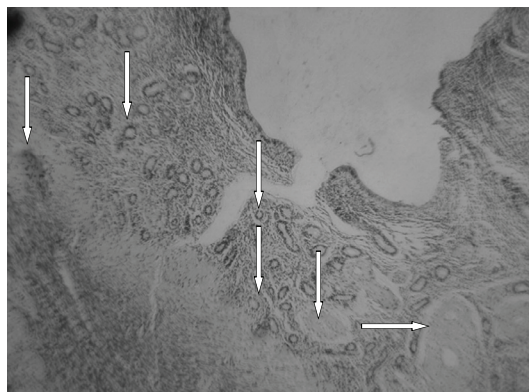
**Table 1:** Prevalence of the Gross abnormalities of the Slaughtered Cow genitalia in Lafenwa abattoir

Abnormality	Number (percentage)	Remarks
Ovarian Hypoplasia	14 (7.0)	Left and Unilateral
Ovarian Tumour	29 (14.5)	Left and Unilateral
Endometritis	57 (28.5)	Found in the uterine horns
Vulvae tick infestation	100 (50.0)	Ticks isolated were identified to be <i>Amblyomma</i> sp., <i>Boophilus</i> sp., <i>Rhipicephalus</i> sp. (Hendrix and Robinson, 2006).
<b>Total</b>	<b>200 (100)</b>	

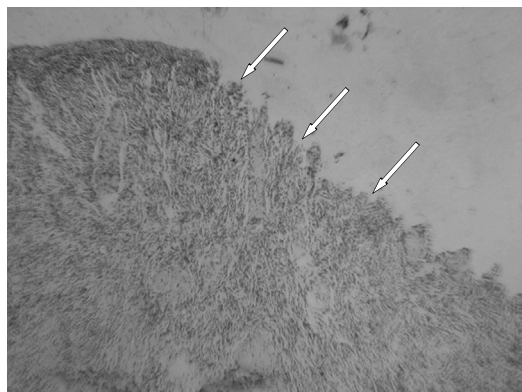
**Table 2:** Frequency distribution of gravid and non-gravid uteri encountered during the abattoir survey

Uterine State	Number (Percentage)	Remarks
Gravid	71 (35.5)	Found in the left horn
Non Gravid	129 (64.5)	-
<b>Total</b>	<b>200 (100)</b>	

**Plate 1:** Gross appearance of an abnormal ovary (ovarian tumour)**Plate 3:** Bovine ovarian hypoplasia (upper) and normal functional ovary (lower)**Plate 2:** Arrow showing the points of attachment of vulvae tick infestation**Plate 4:** Picture showing mucosal folds of the endometrium with glistening mucoid material



**Plate 5:** Histological section showing endometritis accompanied by tissue reactions



**Plate 6:** Histological section showing desquamated epithelium in the uterus of a cow

of the year the study was carried out. Tick parasitism is known to be high during rainy season. The reproductive implication of this is seen in the cows being infertile to natural service as a result of bruising of the vulva arising from the tick infestation (Noakes *et al.*, 2001).

The incidence value obtained for ovarian tumour in this work (14.5%) is higher than that reported by Al- Dahash and David (1977) which was 0.23% whilst the occurrence of endometritis as reported in this study is in concordance with the findings of Noakes *et al.*, (2001).

It may be concluded that the results of the present survey could provide baseline information on the prevalence of bovine reproductive diseases in Lafenwa, Abeokuta, Ogun state, Southwest Nigeria.

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# **BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA**

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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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- The research meets all applicable standards for the ethics of experimentation and research integrity. Research to be published must have been conducted to the highest ethical standards. A brief description of the most common of these is described in our Editorial and Publishing Policies.

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## Types of contribution

Full papers providing accounts of original work: Research containing significant new findings. The material presented should be original and not have been published elsewhere, except in a preliminary form. Papers will be reviewed by three referees familiar with the subject matter of the paper. Revisions are likely to be expected. Short Communications: are intended to provide quick

Publication of highly relevant and interesting information. Manuscripts will be peer reviewed by two reviewers and the Editor. Review Articles: should cover subjects falling within the scope of the bulletin, which are of active current interest. Papers need not contain original work or ideas. They will be reviewed for completeness, accuracy, style and suitability of content by referees familiar with the subject and the Editor-in-Chief. Revisions may be requested.

Editorial: articles are short articles describing news about the bulletin or the opinion of the editor-in-chief, the publisher or a guest editor of a thematic series.

Letters to the Editor: the bulletin welcomes letters to the editor. The purpose of Letters to the Editor is to provide a forum for positive and constructive views on articles and matters published in the bulletin. Letters to the Editor must not exceed 300 words. Letters to the editors include technical reports from countries or projects.

Key notes: The editor will, from time to time, invite selected key figures in the field of animal health and production for key notes on specific topics. These invited papers are not subject to revision.

Book Reviews: are accepted and should provide an overview of the work's contents and a critique of the work's value. Book reviews should be limited to 1000 words.

Conference Proceedings: Special Issues of the bulletin may be dedicated to publication of proceedings of key meetings/conferences

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.
2. Use Times New Roman 12 point font for all text except for tables and figures where Times New Roman 10 font should be used.
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.
6. Place page numbers in the lower right hand corner of your manuscript.
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.
10. References should take the following form: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by 'et al.'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works. Examples: Abayomi (2000), Agindotan et al., (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993, 1995), (Kumasi et al., 2001)

Please ensure that references in the text exactly match those in the manuscript's reference list. Check each reference in the text to see that you have the complete citation in the reference section of the paper in the desired style. In the references section, references are listed in alphabetical order.

### Examples of References

- Journal Articles: Ouyang D, Bartholic J, Selegue 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 J, 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. Abbreviations, Symbols and Nomenclature

All specifications must be stated according to the S.I. system. Concentrations of chemical solutions are to be given in mol/l. All other concentrations should be given in % (volume or weight). Any abbreviations of chemical, biological, medical or other terms should only be employed when it is certain that they are internationally known. The full name must be stated in brackets when the abbreviation is first used. Names of micro-organisms and zoological names will be printed in italics and should be underlined in the manuscript.

### 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.

1. When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort.
2. All studies using animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

### Revising your article

When you submit a revised version of your article in response to the referees' comments, you must accompany it with a detailed list of the changes made (ignoring typographical errors, but mentioning additional paragraphs, changes to figures, etc) suitable for transmission to the referee. Where changes have been made in response to the referees' remarks it is important to mention

this and indicate where they can be found. You may also wish to send in a second copy of your article with the changes marked or underlined.

You should go through the referees' comments and for each comment mention whether you followed their suggestion or whether you disagree and wish to respond to the comment. If a referee has misunderstood a point, it is not necessarily their fault and may have been caused by ambiguity or lack of clarity in your article which needs to be corrected. Some authors copy out each of the referees' comments in turn and include their response immediately after. In other cases responses can be made referring back to the reports. Finally, please make sure that you send your revised article to us and not simply the original version again. This is a common mistake, especially when authors send in their work electronically. Electronic revised articles should contain all text and graphics files needed to generate the revised version, and not just those files that have changed.

By observing these guidelines you will be assisting the referees, who give up their time to review manuscripts. If you prepare your article carefully, this can save valuable time during the publication process.

### Appeal of Decision

Authors who wish to appeal the decision on their submitted paper may do so by e-mailing the editorial office with a detailed explanation for why they find reasons to appeal the decision.

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One set of proofs will be sent to the author to be checked for printer's errors and should be returned within three days.

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