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EFFECT OF DIETARY ORGANIC AND INORGANIC COPPER SUPPLEMENT ON APPARENT NUTRIENT DIGESTIBILITY, TRACE MINERAL RETENTION, ILEAL MORPHOLOGY AND BLOOD PARAMETERS OF COCKERELS

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Abstract

The effect of dietary copper source and dosage on growth, apparent nutrient digestibility, trace mineral retention, ileal morphology and blood parameters of cockerel chicks were investigated using two hundred and forty (240) day-old chicks arranged in a 2 × 3 factorial arrangements involving 2 Cu sources (copper sulphate (CuSO₄), copper proteinate (Cu P)) supplemented at 3 levels (0, 50 and 100 mg/kg). There were 6 treatment groups of 40 birds each replicated 5 times with 8 birds per replicate. Birds fed diet supplemented with CuSO₄ and those fed diet supplemented with 100 mg/kg Cu from CuP showed improved (P<0.05) daily feed intake. Chicks fed diet supplemented with 100 mg/kg Cu sourced from CuP recorded the highest (P<0.05) apparent crude protein digestibility and Fe retention. Dietary supplemented with CuP showed increased (P<0.05) Cu, Zn and Fe retention when compared to their counterparts fed diet supplemented with CuSO₄. Packed cell volume (PCV), total serum protein, serum glucose and serum Cu concentration increased (P<0.05) while serum uric acid, serum triglyceride and serum cholesterol concentration reduced (P<0.05) with increasing dietary Cu dosage. Highest (P<0.05) serum glucose, serum Cu, least (P<0.05) serum triglyceride, cholesterol and creatinine concentration were obtained with birds fed diet supplemented with 100 mg/kg Cu sourced from CuP. Dietary supplementation with CuP resulted in increased (P<0.05) villi height when compared with birds fed diet supplemented with CuSO₄. Dietary supplementation of up to 100 mg/kg Cu sourced from CuP is recommended for improved feed intake, apparent nutrient digestibility, trace mineral retention, serum biochemistry and gut morphology of cockerel chicks.

Keywords: Apparent nutrient digestibility, cockerel chicks, copper proteinate, ileal morphology, trace mineral retention

EFFET DE LA SUPPLÉMENTATION EN CUIVRE ORGANIQUE ET INORGANIQUE SUR LA DIGESTIBILITÉ APPARENTE DES NUTRIMENTS, LA RÉTENTION DES OLIGOÉLÉMENTS, LA MORPHOLOGIE DE L'ILÉON ET LES PARAMÈTRES SANGUINS DE JEUNES COQS

Résumé

L'effet de la source et du dosage de cuivre diététique sur la croissance, la digestibilité apparente des nutriments, la rétention des oligoéléments, la morphologie de l'iléon et les paramètres sanguins de poussins mâles a fait l'objet d'une étude portant sur deux cent quarante (240) poussins d'un jour, répartis dans des dispositifs factoriels 2 × 3 utilisant 2 sources de cuivre [(à savoir le sulfate de cuivre (CuSO₄) et le protéinate de cuivre (Cu P)] donné comme supplément à 3 niveaux (0 ; 50 ; et 100 mg / kg). Six (6)

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groupes de traitement de 40 oiseaux chacun ont été constitués, et ont été répétés 5 fois avec 8 oiseaux par répétition. Les oiseaux soumis au régime alimentaire supplémenté avec du CuSO₄ et ceux soumis au régime alimentaire supplémenté avec 100 mg / kg de CuP ont montré une amélioration ($P < 0,05$) de la prise alimentaire quotidienne. Les poussins soumis au régime supplémenté avec 100 mg / kg de Cu provenant du CuP ont enregistré la plus forte ($P < 0,05$) digestibilité apparente de protéines brutes et la plus forte rétention de Fe. Le régime supplémenté avec du CuP a conduit à une augmentation ($P < 0,05$) de la rétention de Cu, de Zn et de Fe par rapport au régime supplémenté avec du CuSO₄. L'hématocrite (PCV), les protéines sériques totales, les taux de glucose sanguin et de Cu sérique ont augmenté ($P < 0,05$), tandis que les taux d'acide urique sérique, de triglycérides et de cholestérol sériques ont diminué ($P < 0,05$) à la suite de l'augmentation de la dose de Cu dans l'alimentation. Le taux le plus élevé ($P < 0,05$) de glucose sérique et de Cu sérique, les taux les plus bas ($P < 0,05$) de triglycérides sériques, de cholestérol et de créatinine ont été notés chez les oiseaux soumis au régime supplémenté avec 100 mg / kg de Cu provenant du CuP. Les régimes alimentaires supplémentés avec du CuP ont entraîné une augmentation ($P < 0,05$) de la hauteur des villosités par rapport au régime supplémenté avec du CuSO₄. La supplémentation jusqu'à 100 mg / kg de Cu provenant du CuP est recommandée pour l'amélioration de la prise alimentaire, la digestibilité apparente des nutriments, la rétention des oligoéléments, la biochimie sérique et la morphologie de l'iléon de poussins mâles.

Mots-clés : digestibilité apparente des nutriments ; poussins mâles ; protéinate de cuivre ; morphologie de l'iléon ; rétention des oligoéléments

Introduction

Copper (Cu) is an essential micro-element required to maintain the normal activities of metalloenzymes associated with iron metabolism, proper functioning of the immune and cardiovascular system (Close, 1998). Cu is a co-factor in cytochrome oxidase, lysyl oxidase, superoxidase dismutase and ceruloplasmin (Lim and Park, 2006). Minimum daily requirement of Cu for most avian species has been established as 5-8 mg/kg diet. Dietary inclusion of Cu above recommended pharmacological dosage has been reported to further improve growth, feed efficiency, reduce plasma cholesterol and improve lipid metabolism in broiler chickens (Baker *et al.*, 1991; Chowdhury *et al.*, 2004)

Over the years, inorganic Cu salts (like sulphates, carbonates, chlorides and oxides) have been widely used as additives to supply Cu in poultry ration. However, the utilization of inorganic mineral salts have faced serious criticism due to the fact that they break down and form very reactive free ions which sometimes complex with nutrient molecules in the digestive tract thereby making nutrients less digestible and unavailable for absorption (Close, 1998). Hence, the use of organic Cu salts (like Cu proteinate, Cu lysine, Cu methionine) has been advocated with increased potential as

feed additives. Improved nutrient absorption and growth have been reported following dietary inclusion of organic salts than their inorganic counterparts (Baker and Ammerman, 1995). Studies have also shown that the efficacy and bioavailability of inorganic and organic sources of Cu differ significantly (Du *et al.*, 1996). Research studies with pigs have shown improved erythrocyte count and haemoglobin concentration with piglets farrowed from sows fed diets supplemented with iron proteinate when compared with piglets farrowed from sows fed with inorganic iron sources (Egeli *et al.*, 1996). The aim of this experiment is to investigate the effect of dietary organic and inorganic copper supplement on apparent nutrient digestibility, trace mineral retention, ileal morphology and blood parameters of cockerels.

Materials and Methods

The research work was carried out at the Poultry Unit of the Teaching and Research Farm Directorate, Federal University of Agriculture, Abeokuta, Nigeria (Latitude 7° 13' 49.46"N and Longitude 3° 26' 11.98"E). Average temperature of 28.5°C and mean annual rainfall of 1037mm existed during the experimental period.

Cu sources

Cu proteinate (CuP) and Cu sulphate (CuSO₄) were used in the current study as organic and inorganic Cu salts, respectively. CuP containing 10% Cu (Bioplex Cu) was obtained commercially (from Alltech® Inc., USA). Feed grade Cu sulphate pentahydrate (CuSO₄·5H₂O) containing 25% Cu was also obtained commercially (Lucaris Ltd, Lagos, Nigeria).

Experimental birds and management

A total of two hundred and forty day-old cockerel chicks sourced from a commercial hatchery were assigned to 30 pens of similar sizes with each pen (of dimension 2.0m × 1.5m) housing 8 birds. Five (5) pens were assigned to each treatment making a total of 6 treatment groups in all. Brooding was done for 21 days. During brooding, temperature was controlled at 36°C for the first 0 to 2 days and then gradually reduced by 2.5°C per week to a final temperature of 28.5°C at the last week of brooding. Temperature was maintained at ambient condition (28.5°C) throughout the study period. The birds were managed intensively in a deep litter pen littered with dried wood shavings. The experiment lasted for 56 days.

Dietary treatments

A basal diet was formulated to contain 32.65 mg/kg basal Cu (Table 1). Six experimental diets were subsequently formulated using the basal diet in a 2 × 3 factorial arrangements of 2 sources of Cu salts (CuSO₄, CuP) supplemented at 3 levels of inclusion (0, 50 and 100 mg/kg). Supplemental Cu salts were included in the basal diet to formulate the experimental diets. Birds contained in each group were assigned to the respective experimental diets. Diets were formulated to meet the nutrient requirements of 0 - 56 days cockerels (NRC, 1994). Feed and water were supplied ad libitum.

Parameters measured

Growth performance

Feed intake and body weight gain was measured on a weekly basis while feed conversion ratio was estimated as the ratio of

feed consumed to weight gained.

Apparent nutrient digestibility and trace mineral retention

At day 56, two birds per pen (n = 10 birds sampled from each treatment) were randomly selected and housed individually in wired-floor metabolic cages (60 cm long × 45 cm wide × 60 cm high per cage). Three days acclimatization period were allowed prior to 4 days collection period. Birds were fed with the quantity of feed which matched previous daily feed intake. Excreta voided were collected and dried overnight at 60°C. Diets and dried excreta samples were analyzed for proximate constituents (AOAC, 1990). Samples of dried excreta and experimental diets were ignited at 400 °C for 4 h in a muffle furnace. The ash was reconstituted using wet-ashing procedure while trace mineral (Cu, Zn, Mn and Fe) analysis was done using a Perkin Elmer Optima 4300DV ICP spectrophotometer (Perkin Elmer, Beaconsfield, UK).

Ileal morphology

At the end of the study, two birds per pen (n=10 birds per treatment) were selected (based on their final liveweight) and slaughtered. Gastrointestinal tract were dissected aseptically from each of the selected bird and ligated into intestinal segments. The ileum was taken distally from Meckel's diverticulum to the caecal junction. Ileum samples (each about 0.5 cm in length) were taken at the mid-point of ileum for histological measurements (Goodlad *et al.*, 1991). Samples were kept for 24 h in acetic acid/ethanol (25/75, v/v), followed by rehydration in a bath of ethanol/water (50/50, v/v) and then in distilled water. Thereafter, samples were stained with the Feulgen reaction: hydrolysis in 1N HCL at 60°C for 6 min, rinsed thrice with distilled water and stained with Schiff reagent for 30 min. Intestinal villi with their crypts were separated under a dissecting microscope.

Blood parameters

At 56th days, blood samples (2.5 ml each) were collected from the brachial wing vein of 2 birds per pen (n = 10 per treatment) into vials containing ethylene diamine tetra-

acetate for the determination of haematological indices while another set of blood was

Table 1. Gross composition of basal diet

Ingredients	Composition
Maize	48.00
Soybean meal	15.00
Groundnut cake	11.00
Fish meal (72% Crude protein)	2.00
Wheat offal	19.20
Bone meal	1.50
Oyster shell	2.50
Salt (NaCl)	0.25
*Premix (Cu free)	0.25
Methionine	0.20
Lysine	0.10
Total	100.00
Determined Analysis (DM basis)	
Crude protein (%)	22.04
Crude fibre (%)	4.26
Ether extract (%)	4.01
Metabolizable energy (MJ/kg) ^a	11.50
Calcium (%) ^a	1.45
Phosphorus (%) ^a	0.54
Lysine (%) ^a	0.91
Methionine (%) ^a	0.54
Cu level in basal diet (mg/kg) ^a	32.65

Vitamins/mineral premix (Godomix©) included at the rate of 2.5 kg per ton of feed contained the following: Vitamin A: 3,200,000 IU, vitamin D: 640,000 IU, vitamin E: 2000 mg, vitamin k3: 800 mg, vitamin B1: 2000 mg, vitamin B2: 6000 mg, vitamin B6: 5000 mg, vitamin B12: 25 mg, niacin: 6000 mg, panthotenic acid: 20,000 mg, folic acid: 1000 mg, biotin: 8 mg, manganese: 30,000 mg, iron: 20,000 mg, zinc: 20,000 mg, copper: nil, cobalt: 80 mg, iodine: 480 mg, selenium: 40 mg, choline: 800,000 mg BTH: 25,000 mg.

Supplementary dietary Cu was added to the basal diets at 50, 100 and 150 mg/kg Cu concentrations.

^aCalculated value

collected into heparinised tubes for serum thiocyanate determination. Haemoglobin concentration (Hb) was estimated using the cyanmethaemoglobin method (Cannan, 1958). Packed cell volume (PCV), total erythrocyte

count and white blood cell count (WBC) of blood samples were determined in a Wintrobe haematocrit tube (Schalm *et al.*, 1975). Differential leucocyte counts (heterophils, lymphocytes, eosinophils, monocytes) were carried out on blood smears stained with May-Grunwald-Giemsa stain.

Plasma was harvested by centrifuging the whole blood samples at 3,000 rpm for 15 min. The total serum protein, albumin and globulin were determined using bromocresol purple method (Varley *et al.*, 1980). Serum creatinine (Bousnes and Tausky, 1945) and serum uric acid concentration (Wootton, 1964) were determined according to standard methods. Plasma Cu was determined using the atomic absorption spectrophotometer (Perkin Elmer A Analyst 100). Serum enzymes (alanine transaminase (ALT) and aspartate serum transaminase (AST)) were analysed spectrophotometrically using RandoXR diagnostic cholesterol kit. The serum cholesterol was estimated using the commercial kits (Qualigens India. Pvt. Ltd., Catalogue number 72201-04)

Statistical analysis

Data generated were subjected to analysis of variance using the general linear model procedure of the SAS (1991) to determine the main effect of Cu dosage, source and their interaction (Cu dosage × Cu source). Significant differences were considered at $P < 0.05$.

Result

Growth performance, apparent nutrient digestibility and trace mineral retention

The main effect of Cu dosage and source on growth performance, apparent nutrient digestibility and trace mineral retention of cockerel chicks is shown in Table 2. Dietary supplementation of 100 mg/kg Cu had the highest daily feed intake ($P < 0.05$) and total Cu intake ($P < 0.0001$). Total Cu intake increased ($P < 0.0001$) with increasing dietary Cu dosage supplemented. Birds fed diet supplemented with CuSO₄ had higher ($P < 0.05$) daily feed intake and total Cu intake than those fed with CuP.

Dietary supplementation with Cu irrespective of dosage showed improved ($P<0.05$) apparent dry matter digestibility. Apparent crude protein digestibility increased ($P<0.05$) while crude fibre digestibility reduced ($P<0.05$) with increasing dosage of Cu. Dietary supplementation with CuP showed improved ($P<0.05$) apparent crude protein digestibility when compared to their counterparts fed diet supplemented with CuSO_4 . Control-fed birds had the least ($P<0.05$) Fe retention and highest ($P<0.05$) Zn retention. Dietary supplementation with Cu irrespective of dosage showed reduced ($P<0.05$) Zn retention. Fe retention improved ($P<0.05$) with increasing dosage of Cu. Birds fed diet supplemented with CuP showed increased ($P<0.05$) Cu, Zn and Fe retention when compared with birds fed diet supplemented with CuSO_4 .

The interaction effect of dosage and source of Cu on growth performance, apparent nutrient digestibility and trace mineral retention of cockerel chicks is shown in Table 3. Birds fed diet supplemented with CuSO_4 (irrespective of dosage) and those fed diet supplemented with 100 mg/kg Cu from CuP showed the highest ($P<0.05$) daily feed intake. Irrespective of Cu source, Cu intake increased ($P<0.01$) with increasing dietary dosage of Cu. Birds fed diet containing 100 mg/kg Cu from CuP recorded the highest overall ($P<0.05$) total Cu intake and apparent crude protein digestibility. Reduced ($P<0.05$) apparent crude protein digestibility was obtained with birds fed control diet and those fed diet supplemented with CuSO_4 .

Birds fed diet supplemented with CuP (irrespective of Cu dosage) showed higher ($P<0.05$) Cu retention than those fed diet supplemented with CuSO_4 . Birds fed diet supplemented with 50 mg/kg Cu from CuSO_4 showed the least ($P<0.05$) Cu and Zn retention. Birds fed diet supplemented with 100mg/kg Cu from CuP had the highest ($P<0.05$) Fe retention. Birds fed control diet and those fed diet supplemented with CuSO_4 had low ($P<0.05$) Fe retention values.

Blood parameters and ileal morphology

The main effect of Cu dosage and

source on the blood parameters and ileal morphology of cockerel chicks is shown in Table 4. Packed cell volume (PCV), total serum protein, serum glucose and serum Cu concentration increased ($P<0.05$) while serum uric acid, serum triglyceride and serum cholesterol concentration reduced ($P<0.05$) with increasing Cu dosage. Dietary supplementation with CuP showed increased ($P<0.05$) serum glucose, triglyceride and serum Cu while supplementation with CuSO_4 resulted in increased ($P<0.05$) total serum glucose, serum globulin, serum uric acid and cholesterol concentration. Cu source showed no effect ($P>0.05$) on PCV, haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), SGOT, SGPT and serum creatinine concentration.

Villi height of the ileum samples increased ($P<0.05$) with increasing Cu dosage. Villi depth and villi/crypt ratio were not affected ($P>0.05$) by the main effect of Cu dosage. Dietary supplementation with CuP showed increased ($P<0.05$) villi depth when compared with birds fed diet supplemented with CuSO_4 . Villi depth and villi/crypt ratio were not affected ($P>0.05$) by Cu source supplemented.

The interaction effect of Cu dosage and source on blood parameters and ileal morphology of cockerel chicks is shown in Table 5. Dietary supplementation with 100 mg/kg Cu sourced from CuP recorded the highest ($P<0.05$) serum glucose and serum Cu concentration, least ($P<0.05$) serum triglyceride, cholesterol and creatinine concentration. Chicks fed control diet recorded the least ($P<0.05$) serum glucose concentration. All birds fed diet supplemented with CuP showed reduced ($P<0.05$) serum uric acid concentration.

Highest ($P<0.05$) ileal villi height was obtained with birds fed diet supplemented with 100 mg/kg Cu sourced from CuP. All birds fed diet supplemented with CuP (irrespective of Cu dosage) showed higher ($P<0.05$) villi height than those birds fed diet supplemented with CuSO_4 . The crypt depth and villi height/crypt depth ratio were not affected ($P>0.05$) by the interaction effect of Cu dosage and source in this study.

Table 2: Main effect of Cu levels and source on growth performance, apparent nutrient digestibility and trace mineral retention of cockerel chicks fed diet supplemented with copper sulphate or copper proteinate

Parameters	Main effect of Cu levels				P-value	Main effect of Cu source			
	0	50mg/kg	100mg/kg	SEM		CuSO ₄ ·5H ₂ O	CuP	SEM	P-value
Initial weight (g/bird)	37.66	37.42	37.00	1.02	0.195	37.39	37.33	0.90	0.100
Daily feed intake (g/bird)	39.19 ^b	41.00 ^b	45.12 ^a	4.02	0.033	43.38 ^a	40.15 ^b	3.79	0.044
Total Cu intake (mg/bird)	71.67 ^c	132.62 ^b	334.93 ^a	22.75	<0.0001	202.77 ^a	194.79 ^b	32.65	0.041
Final weight/bird (g)	656.67	680.02	696.67	76.55	0.066	673.33	684.33	77.20	0.063
Daily weight gain (g/bird)	11.05	11.46	11.78	0.92	0.065	11.51	11.34	0.77	0.077
Feed conversion ratio	3.55	3.58	3.83	0.09	0.061	3.77	3.53	0.05	0.099
Apparent nutrient digestibility (%)									
Dry matter	80.79 ^b	84.89 ^a	85.14 ^a	9.55	0.040	83.72	83.47	1.90	0.095
Crude protein	70.10 ^c	71.92 ^b	76.49 ^a	9.01	0.029	70.44 ^b	75.23 ^a	7.02	0.028
Ether extract	63.01	64.79	66.23	2.77	0.039	64.72	64.63	1.22	0.090
Crude fibre	60.45 ^a	56.16 ^b	50.86 ^c	7.02	0.022	54.81	56.63	2.95	0.065
Trace mineral retention (%)									
Cu	28.30 ^a	26.55 ^{ab}	26.15 ^b	4.02	0.033	24.90 ^b	29.10 ^a	3.99	0.029
Zn	32.80 ^a	23.45 ^b	25.01 ^b	3.99	<0.0001	24.20 ^b	29.97 ^a	4.11	0.022
Mn	21.98	21.93	21.93	1.01	0.095	21.80	22.08	1.04	0.077
Fe	19.36 ^c	22.00 ^b	23.85 ^a	3.33	0.027	19.37 ^b	24.11 ^a	3.29	0.020

^{a,b,c}Means in the same column with different superscript differ significantly ($P < 0.05$).

Table 3: Interaction effect of Cu levels and source on growth performance, apparent nutrient digestibility and trace mineral retention of cockerels fed diet supplemented with copper sulphate or copper proteinate

Parameters	CuSO ₄ ·5H ₂ O			SEM	Cu Proteinate			P-value
	0	50mg/kg	100mg/kg		0	50mg/kg	100mg/kg	
Initial weight (g/bird)	37.67	37.50	37.00	1.04	37.65	37.33	37.00	0.122
Daily feed intake (g/bird)	42.37 ^b	43.07 ^{ab}	44.70 ^a	3.99	36.00 ^d	38.93 ^c	45.53 ^a	0.019
Total Cu intake (mg/bird)	77.38 ^e	199.29 ^c	331.63 ^b	37.22	65.95 ^f	180.18 ^d	338.23 ^a	0.005
Final weight/bird (g)	660.00	673.33	686.67	77.20	653.33	686.70	706.67	0.067
Daily weight gain (g/bird)	11.01	11.56	11.95	0.99	11.08	11.35	11.60	0.055
Feed conversion ratio	3.85	3.73	3.74	0.53	3.25	3.43	3.92	0.099
Apparent nutrient digestibility (%)								
Dry matter	81.43 ^e	84.80 ^c	84.98 ^b	8.57	80.144 ^e	84.99 ^b	85.29 ^a	0.041
Crude protein	69.56 ^c	70.17 ^c	71.60 ^{bc}	8.06	70.64 ^c	73.66 ^b	81.38 ^a	0.033

Parameters	CuSO ₄ ·5H ₂ O			Cu Proteinate			SEM	P-value
	0	50mg/kg	100mg/kg	0	50mg/kg	100mg/kg		
Ether extract	63.11	64.72	66.33	62.91	64.85	66.12	2.44	0.062
Crude fibre	58.38 ^a	56.14 ^b	49.90 ^d	62.51 ^a	56.17 ^c	51.82 ^e	7.02	0.005
Trace mineral retention (%)								
Cu	28.40 ^b	24.20 ^c	22.10 ^d	28.20 ^b	28.90 ^{ab}	30.20 ^a	4.66	0.043
Zn	32.60 ^a	20.20 ^d	19.80 ^d	33.00 ^a	26.70 ^c	30.22 ^b	5.02	0.019
Mn	21.95	21.55	21.90	22.00 ^c	22.30	21.95	1.66	0.020
Fe	19.50 ^c	19.40 ^c	19.20 ^c	19.22 ^c	24.60 ^b	28.50 ^a	4.24	0.022

^{a,b,c}Means in the same column with different superscript differ significantly (P<0.05).

Table 4: Main effect of Cu levels and source on blood parameters and ileal morphology of cockerel chicks fed diet supplemented with copper sulphate or copper proteinate

Parameters	Main effect of Cu levels					Main effect of Cu source			
	0	50mg/kg	100mg/kg	SEM	P-value	CuSO ₄ ·5H ₂ O	CuP	SEM	P-value
Blood parameters									
Packed cell volume (%)	33.50 ^c	35.50 ^b	37.50 ^a	3.22	0.044	35.33	35.67	0.96	0.120
Haemoglobin (g/dl)	11.17	11.90	12.45	0.90	0.150	11.77	11.91	0.92	0.099
Red blood cell (x10 ¹² /L)	2.04	2.45	2.69	0.80	0.110	1.75	2.37	1.02	0.077
White blood cell (x10 ⁹ /L)	5.77	5.89	6.29	1.01	0.095	5.99	5.97	0.98	0.108
Glucose (mg/dl)	92.34 ^c	100.34 ^b	107.00 ^a	9.04	0.022	95.56 ^b	104.22 ^a	9.19	0.023
Total protein (g/l)	40.52 ^c	42.02 ^b	43.79 ^a	3.77	0.029	44.73 ^a	39.48 ^b	4.01	0.040
Albumin (g/l)	26.72 ^b	29.92 ^a	29.39 ^a	3.64	0.042	29.67	26.34	4.33	0.190
Globulin (g/l)	13.80	14.10	14.40	1.24	0.085	15.07 ^a	13.13 ^b	3.05	0.029
SGOT (IU/L)	78.00 ^a	76.50 ^{ab}	75.00 ^b	6.21	0.044	76.00	77.00	1.00	0.097
SGPT (IU/L)	4.95	4.75	4.55	0.75	0.099	4.47	5.03	1.01	0.077
Uric acid (mg/dl)	3.20 ^a	2.40 ^b	1.10 ^c	1.99	0.041	2.97 ^a	1.50 ^b	1.94	0.042
Creatinine (mg/dl)	0.40 ^a	0.34 ^{ab}	0.20 ^b	0.22	0.040	0.37	0.26	0.04	0.199
Triglyceride (mg/dl)	120.00 ^a	115.00 ^b	108.50 ^c	8.65	0.022	89.33 ^b	94.00 ^a	7.97	0.044
Cholesterol (mg/dl)	151.50 ^a	149.00 ^b	145.50 ^c	11.02	0.029	152.00 ^a	145.33 ^b	10.42	0.023
Copper (µmol/l)	13.62 ^c	15.78 ^b	18.62 ^a	7.85	0.043	12.79 ^b	19.22 ^a	7.08	0.041
Ileal Morphology									
Villi height (µm)	603.00 ^c	627.50 ^b	633.50 ^a	40.50	0.040	608.00 ^b	634.30 ^a	42.75	0.042
Crypt depth (µm)	99.50	100.50	101.50	6.05	0.097	99.30	101.70	5.44	0.099
Villi height/Crypt depth	6.06	6.24	6.24	0.42	0.090	6.12	6.24	0.50	0.155

^{a,b,c}Means in the same column with different superscript differ significantly (P<0.05).

Table 5: Interaction effect of Cu levels and source on blood parameters and ileal morphology of cockerel chicks fed diet supplemented with copper sulphate or copper proteinate

Parameters	CuSO ₄ ·5H ₂ O			Cu Proteinate			SEM	P-value
	0	50mg/kg	100mg/kg	0	50mg/kg	100mg/kg		
Blood parameters								
Packed cell volume (%)	34.00	35.00	37.00	33.00	36.00	38.00	1.92	0.064
Haemoglobin (g/dl)	11.30	11.70	12.30	11.03	12.10	12.60	0.99	0.085
Red blood cell (×10 ¹² /L)	2.07	2.40	2.77	2.00	2.50	2.60	0.88	0.069
White blood cell (×10 ⁹ /L)	5.73	5.87	6.37	5.80	5.90	6.20	1.06	0.075
Glucose (mg/dl)	91.00 ^d	95.67 ^c	100.00 ^{bc}	93.67 ^{cd}	105.00 ^b	114.00 ^a	10.02	<0.0001
Total protein (g/l)	43.20 ^b	45.10 ^{ab}	45.90 ^a	37.83 ^c	38.93 ^c	41.67 ^{ab}	4.07	0.040
Albumin (g/l)	28.40 ^b	30.00 ^{ab}	30.60 ^a	25.03 ^c	25.83 ^c	28.17 ^{ab}	3.93	0.042
Globulin (g/l)	14.80 ^{ab}	15.10 ^{ab}	15.30 ^a	12.80 ^d	13.10 ^c	13.50 ^b	3.03	0.029
SGOT (IU/L)	77.00 ^{ab}	76.00 ^{ab}	75.00 ^{ab}	79.00 ^a	77.00 ^{ab}	75.00 ^b	5.94	0.044
SGPT (IU/L)	4.70	4.50	4.20	5.20	5.00	4.90	0.92	0.099
Uric acid (mg/dl)	3.80 ^a	3.80 ^a	1.30 ^{bc}	2.60 ^b	1.00 ^c	0.90 ^c	1.94	0.041
Creatinine (mg/dl)	0.43 ^a	0.37 ^{ab}	0.30 ^b	0.37 ^{ab}	0.30 ^b	0.10 ^f	0.20	0.033
Triglyceride (mg/dl)	130.00 ^a	127.00 ^b	121.00 ^c	110.00 ^d	103.00 ^e	96.00 ^f	9.84	0.033
Cholesterol (mg/dl)	155.00 ^a	152.00 ^b	149.00 ^{bc}	148.00 ^{bc}	146.00 ^c	142.00 ^d	10.66	0.041
Copper (µmol/l)	11.02 ^e	13.15 ^d	14.21 ^d	16.22 ^c	18.41 ^b	23.02 ^a	4.69	0.022
Ileal Morphology								
Villi height (µm)	602 ^d	610 ^c	612 ^c	603 ^d	645 ^b	655 ^a	49.70	0.035
Crypt depth (µm)	99	98	101	100	103	102	6.55	0.077
Villi height/Crypt depth	6.08	6.22	6.06	6.03	6.26	6.42	0.55	0.062

^{a,b,c,d,e,f} Means in the same column with different superscript differ significantly ($P < 0.05$).

Discussion

Improved feed intake of cockerel chicks obtained in the current study following dietary supplementation with Cu corroborated previous study which reported increased feed intake of broiler chicks fed diet supplemented with copper salts (Chowdhury *et al.*, 2004). Previous findings also confirmed improved feed efficiency following dietary supplementation of Cu at higher pharmacological levels (Chori and Park, 1989). High intake of Cu recorded with birds fed diet supplemented with 100 mg/kg Cu could be attributed to the increased feed consumption obtained at this level of inclusion.

The absence of significant influence on growth response of birds following inclusion of varying dosage of copper salts in this study agreed with the previous findings which showed that growth response of chicks were not affected by Cu source, dosage and their interaction (Jegade *et al.*, 2011). Lack of response on growth in the present study may be due to the short-term feeding trial (28 days) of Cu inclusion as reported in related study (Ewing *et al.*, 1998).

Improved apparent dry matter and crude protein digestibility of cockerel chicks obtained in this study following dietary supplementation of Cu agreed with Wang *et al.*

(2010) that Cu stimulates increased activities of endogenous enzymes, improved intestinal microbiota, increase nutrient digestibility and improve gastrointestinal physiology. Dietary supplementation of Cu at slightly higher dosage (above recommended) stimulated small intestinal lipase, phospholipase, improved apparent fat digestibility and apparent nitrogen retention of pigs (Luo and Dove, 1996). Improved apparent crude protein digestibility obtained with chicks fed diet supplemented with 100 mg/kg Cu from CuP confirmed that the organic salt of Cu at this inclusion level showed a better protein absorption and digestion.

Increased Cu retention and reduced Zn retention obtained in this study following dietary supplementation of Cu agreed with previous reports that Cu and Zn mutually hamper the absorption of each other (Van Campen and Scaife, 1967). Improved Fe and Cu retention obtained in the current study following dietary supplementation of Cu corroborated previous study which reported that Cu deficiency is triggered under situation of reduced Fe utilization (Hart *et al.*, 1928). Defective Fe metabolism in Cu deficient animals has been linked with reduced ferroxidase activity in the blood. Iron is released from the liver and absorbed as Fe(II) while transferrin transports Fe in blood and binds only to Fe(III). The oxidation of Fe(II) to Fe(III) is catalyzed by ferroxidase enzymes which utilizes Cu (Frieden and Hsieh, 1976). Elevated levels of dietary Cu increased ferroxidase activity and thus caused a shift in equilibrium that increased Fe absorption.

Improved Cu, Zn and Fe retention obtained in birds fed diet supplemented with CuP when compared with those fed with CuSO₄ implied greater absorption and retention of these minerals from organic salts (Brown and Zeringe, 1994). Improved absorption of Cu from Cu-methionine complex was reported when compared to diets supplemented with CuSO₄ (Paik *et al.*, 1999). Highest Fe retention obtained with chicks fed diet containing 100mg/kg Cu from CuP suggested improved retention of Fe using organic Cu salts at this dosage. The mechanism underlining the improved

absorption and retention of Cu from organic Cu salts may be due to the unique coordination chemistry of organic compound which permits the formation of highly soluble and chemically stable products that resist interaction with antagonists in the gut (Brown and Zeringe, 1994).

Supplemental Cu in the current study showed no negative clinical symptoms of toxicity on the birds. However, increased plasma Cu concentration obtained with birds fed diet supplemented with CuP implied higher bioavailability of Cu from organic salts than the inorganic salts. Higher plasma Cu levels was also reported with sheep supplemented with Bioplex® copper than those fed with copper sulfate (Ryan *et al.*, 1982). Increased PCV of chicks fed diet supplemented with Cu showed indications of improved nutritional status and absence of nutritional anaemia while increased serum glucose suggested indications of higher energy availability (Keir *et al.*, 1982; Makarski and Polonis, 2001).

High total serum protein concentration obtained with increased Cu dosage in this study indicated improved utilization of dietary protein (Church and Pond, 1988). High serum glucose obtained for chicks fed with CuP when compared with those fed with CuSO₄ indicated higher available dietary energy for chicks fed with organic Cu salt. Reduced serum uric acid concentration obtained for birds fed diet supplemented with CuP implied efficient protein utilization and reduced deamination (Oduguwa and Ogunmodede, 1995). The high level of Cu occasioned by higher bioavailability of Cu-P compared to CuSO₄ may have caused the Cu atom to replace the Fe atom in Xanthine oxidase, resulting in a low uric acid level observed for birds fed Cu-P. High Cu nutrition has been implicated in partial inhibition of xanthine oxidase, an iron–molybdenum enzyme (Leslie, 1980).

The least serum triglyceride, cholesterol and creatinine concentration obtained with birds fed with 100 mg/kg CuP confirmed that organic Cu salt is more effective in reducing serum cholesterol and triglyceride than inorganic salt. Reduced plasma triglyceride concentration was reported with broilers fed

with 250 mg/kg Cu from organic source than those from inorganic source (Bakalli *et al.*, 1995). Pullet chicks fed diet supplemented with CuP also showed reduced serum cholesterol and triglyceride concentration when compared with those fed with CuSO₄ (Jegade *et al.*, 2011). The mechanism whereby serum cholesterol reduced following Cu supplementation was attributed to the fact that Cu regulates cholesterol biosynthesis by reducing hepatic glutathione concentration and changing oxidised glutathione ratio, which decreased the activity of 3-hydroxyl-3-methylglutaryl Co- (HMG-CoA) reductase (Kim *et al.*, 1992; Konjufca *et al.*, 1997).

Increased villi height obtained with increasing Cu dosage implied increased intestinal surface area for improved nutrient absorption since supplemental Cu has been reported to stimulate villi cells (Chiou *et al.*, 1997). Inclusion of up to 250 mg/kg Cu in diets for pigs was also reported to stimulate increased villi height and consequently improved growth (Radecki *et al.*, 1992). Highest villi height obtained with chicks fed diet supplemented with 100 mg/kg Cu sourced from CuP indicated that nutrient absorption was at its best with organic salts at this inclusion level of inclusion.

Conclusion

Dietary supplementation of up to 100 mg/kg Cu sourced from copper proteinate is hereby recommended for improved feed intake, apparent nutrient digestibility, trace mineral retention, serum biochemistry and ileal morphology of cockerel chicks.

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GASTROINTESTINAL PARASITES AND MANAGEMENT OF DOMESTIC PIGS (SUS SCROFA) IN NORTH-EASTERN NIGERIA

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Abstract

The study investigated the occurrence of gastrointestinal parasites and the effect of some epidemiological factors on the gastrointestinal parasitism of pigs from southern Adamawa State. Three hundred (300) faecal samples were collected from pigs of both sexes and varying ages from seven local Government areas of Southern Adamawa State and were analyzed coprologically using standard floatation technique. Information on epidemiological variables of the sampled pigs was obtained from farms by means of a well structured questionnaire. An overall infection rate of 84.0% was encountered in this study. Five parasite species (*Ascaris suum*, *Ancylostoma* spp, *Trichuris* spp, *Trichinella spiralis* and *Coccidial oocyst*) were found during the study. There was a statistical significant ($p < 0.05$) variation in infection among the Local Government areas of study. Female pigs were more infected significantly ($p < 0.05$) than their male counterparts. Also, pigs aged > 12 months were significantly ($p < 0.05$) infected with the parasites than the younger pigs. The distribution of the parasites species showed statistically significant variations ($p < 0.05$) based on the zootechnic categories, health status and the history of deworming. This study has shown high occurrence of gastrointestinal parasites among pigs in Adamawa State, with most of the parasites having zoonotic potential.

Keywords: Gastrointestinal parasites, Pig, Adamawa State, Occurrence, Faecal sample

PARASITES GASTROINTESTINAUX DES PORCS DOMESTIQUES (SUS SCROFA) DANS LE NORD-EST DU NIGERIA

Résumé

La présente étude porte sur la présence de parasites gastro-intestinaux et l'effet de certains facteurs épidémiologiques sur le parasitisme gastro-intestinal des porcs dans le Sud de l'État d'Adamawa. Trois cents (300) échantillons de matières fécales ont été prélevés sur les porcs des deux sexes et de différents âges dans sept zones de la collectivité locale du Sud de l'État d'Adamawa et ont été soumis à un examen coprologique utilisant la technique de flottation standard. Les informations sur les variables épidémiologiques des porcs échantillonnés ont été recueillies auprès de fermes, au moyen d'un questionnaire bien structuré. L'étude a noté un taux d'infection global de 84% et a identifié cinq espèces de parasites (*Ascaris suum*, *Ancylostoma* spp, *Trichuris* spp, *Trichinella spiralis* et *Coccidial oocyst*). Elle a relevé une variation statistique significative ($p < 0,05$) de l'infection entre les diverses zones étudiées de la collectivité locale. Les femelles étaient significativement ($p < 0,05$) plus infectées que les mâles. En outre, les porcs âgés de $12 >$ mois étaient significativement ($p < 0,05$) plus infectés par les parasites par rapport aux jeunes porcs. La répartition des espèces de parasites a montré des variations statistiquement significatives ($p < 0,05$) basées sur les catégories zootechniques, l'état de santé et les antécédents de déparasitage. Cette étude a révélé une forte présence de parasites gastro-intestinaux chez les porcs de l'État d'Adamawa, la plupart des parasites ayant un potentiel zoonotique.

Mots-clés : parasites gastro-intestinaux ; porc, État d'Adamawa ; présence ; échantillon fécal

Introduction

About 78% of the Nigeria's pig population is resident in the sub-humid zones of Northern and southern Guinea savannah (Shaibu *et al.*, 1997). Agriculture contributes immensely to the economic base of Adamawa state; employing about 80% of the working population (Seeds 2005). The state's population of pig has been put at 575,000 out of Nigeria's estimated 4.4 million pigs. Pig among other livestock population, in addition, to being the most efficient converter of non-conventional feedstuffs to edible meat, has been shown to be a better carcass yielder dressing out at about 70%, which is far greater than the between 50-55% for cattle, sheep and goats (Ikani and Dafwang, 1995). However, this may be beset with problems of infectious diseases particularly parasitic diseases which is a common problem in all husbandry systems in pig production.

Parasitic diseases are one of the major disease conditions militating against optimal productivity in pig production and this has received little attention compared to parasitism in other food animals like ruminants. This may be because porcine helminthes seldom cause clinical disease and may go unrecognized by farmers and veterinarians (Roepstorff *et al.*, 2011).

Infection with gastrointestinal parasites has been associated with significant economic losses as measured by decreased litter size, poor growth rate, reduced weight gain, organ condemnation at slaughter and death (Nsoso *et al.*, 2000). Furthermore, Theodoropoulos *et al.* (2004) and Knecht *et al.* (2011) observed that, natural infection of pig with intestinal parasites affects meat quality negatively, as differences were observed in some qualitative features of pigs infected with helminthes and those free from worm invasion. Longer invasion period of pigs by helminthes as seen in older animals influences qualitative feature of meat negatively.

Documented evidences exist for the occurrence of gastrointestinal parasitism in pigs from around the world, particularly under husbandry systems deficient of routine veterinary practices. Weng *et al.* (2005)

demonstrated the presence of different intestinal parasites in pigs in China, with some species such as *Ascaris suum* and *Trichuris suis*, isolated from the study having zoonotic potentials. Permin *et al.* (1999) in Ghana and Tamboura *et al.* (2006) in Burkina Faso conducted similar studies. However, in Nigeria, most previous studies (Dipeolu *et al.*, 1982; Weka and Ikeh 2009; Sowemimo *et al.*, 2012; Pam *et al.* 2013) on parasites of pigs have been limited to the southern and north central parts of the country where pigs are reared in large numbers, with little or no information from other parts of the north. The report by Biu *et al.* (2013) on Oesophagostomosis in Michika, northern Adamawa State is one of the few on the occurrence of parasites in pigs in the region. The paucity of information on gastrointestinal parasites of pigs in the Northeastern part of Nigeria particularly Adamawa State, necessitated this study. In addition, the study was aimed at providing information on possible gastrointestinal parasites of zoonotic potential within the region, since pork consumption is a common practice.

Materials and Methods

Study Area

This study was conducted in southern part of Adamawa state comprising seven local Government areas (Ganye, Jada, Toungo, Shelleng, Numan, Demsa and Mayobelwa), from June to September 2012. Adamawa state lies between latitude 7° 28'N and 10° 15' N and longitude 11° 20' and 13° 34' E. It has a landmass of 42,158 km² and is bounded to the north by the Republic of Cameroun, Taraba State to the south and part of Taraba and Gombe States to the west. The state has a tropical climate marked by distinct dry and rainy seasons. The rainy season starts in April and ends in October with average rainfall of 79 and 101 mm in the north and south respectively (Seeds 2005).

Study Animals

The pigs studied were owned by small holder farmers in southern Senatorial District of Adamawa State. There was no previous information on the population of pigs within

the district. However, the State's population has been put at 575,000 (Seeds 2005).

Study design

Sampled Animals and Sampling: A total of thirteen (13) farms were randomly selected from the seven local Government areas of southern Adamawa State. Pigs studied were reared under intensive and semi-intensive systems of management. Three hundred (300) pigs were randomly selected from the identified farms. Prior to faecal sample collection, information on the age, sex, local Government of collection were recorded. The research was approved by the Head, Department of Animal Health Technology, College of Agriculture, Adamawa State and was carried out according to International guiding principles for biochemical research involving animals (C. I. O. M. S, 1985)

Sample Collection, Processing and Parasite Identification

Consents of the participating farms were obtained through their respective Managers and owners. The purpose of the study was fully explained in the local language and understood before sampling commenced.

Faecal samples were collected directly from the rectum of each pig into faecal sample bottle containing Methiolate iodine formalin (MIF) solution as fixative (Pessoa and Martins, 1982). Briefly, the index finger was inserted directly into the rectum after wearing a hand glove and proper restraint of the animal. The faeces collected were transferred to the sample bottle before transportation to the laboratory of the Department of Animal Health Technology, College of Agriculture; Ganye. Sample from each pig was properly labeled and identified by assigning an identification number and was analyzed as described by Urquhart *et al.* (1987).

Administration of Questionnaires

A well structured questionnaire was administered during sample collection. Answers were provided by the Managers of the farms visited. The Questions were explained to individuals in the Local language (Hausa)

for easy comprehension. Components of the questionnaire include; information on recent history of deworming, type of husbandry practice management, health status at the time of sampling and zootechnic category of the the pigs (piglets, dams, winners, sows and Boars).

Data Analysis

Data generated were analyzed using statistical analysis system version 8.0 (SAS, 2003) and the infection rate tested for significance based on local Government area of study, sex and age using analysis of variance (ANOVA). $P < 0.05$ was considered significant throughout the study.

Results

A total of three hundred (300) faecal samples were examined using floatation technique. An overall infection rate of 84.0% was encountered during the study (Table 1). Numan local Government area had the highest prevalence of infection (90.0%), followed by Ganye (87.5%), Jada (86.5%) and Toungo (85.7%) local Government areas. Demsa and Shelleng each had (73.0%) and (70.0%) respectively, while Mayo Belwa had the least infection rate of (69.2%) (Table 1). The prevalence of infection among the various Local Government showed statistically significant differences ($p = 0.016$, $p < 0.05$). Table 2 showed the distribution of the recovered parasites based on the sex of the examined pigs. Female pigs were more significantly infected than their corresponding males ($p = 0.048$, $p < 0.05$). The distribution of the infection rate based on the ages of the examined pigs showed a significant difference ($p < 0.05$) among the various age groups with the rate indicative of age dependent increase (Table 3). Table 4 showed the parasites species and the frequency of recovery from the examined samples. *Ascaris suum* was the most common gastrointestinal parasite encountered in this study with 37.3%, and was followed by *Ancylostoma* spp and *Trichuris* spp with 23.3% and 16.0% respectively. *Trichinella* spp was isolated among 16 (5.0%) pigs, while the least parasite recovered from the study was *Coccidian* oocyst with 2.0%.

Table 1: Prevalence of Gastrointestinal parasitism among pigs from southern Adamawa State, Nigeria

Local Government Area	No. Examined	No.(%) infected	(Mean±SD) Infection
Ganye	112	98(87.5) ^a	64.8±47.8
Toungo	49	42(85.7) ^a	39.6±31.9
Jada	37	32(86.5) ^a	34.8±31.7
Shelleng	10	7(70.0) ^b	24.0±27.7
Numan	40	36(90.0) ^c	36.3±34.0
Demsa	26	19(73.0) ^b	30.3±25.1
Mayo Belwa	26	18(69.2) ^b	30.4±23.4
Total	300	252(84.0)^a	38.5±32.6

Different superscripts in columns differs significantly. $P < 0.016$

Table 2: Prevalence of gastrointestinal parasites of pigs from Southern Adamawa State based on sex

Sex	No. Examined	No.(%) infected	(Mean±SD) Infection
Male	114	90(78.9) ^a	76.0±46.6
Female	186	162(87.0) ^b	176.7±12.2

Different superscripts in columns differs significantly. $P < 0.048$

Table 3: Prevalence of gastrointestinal parasites of pigs from Southern Adamawa State based on age

Age category	No. Examined	No.(%) infected	(Mean±SD) Infection
< 5 months	88	56(63.6) ^a	58.7±28.0
6-12 months	88	72(81.8) ^b	85.3±37.8
> 12 months	124	124(100) ^c	82.7±38.6

Different superscripts in columns differs significantly. $P < 0.032$

Table 4: Distribution of parasite species from Pigs in Southern Adamawa State and their intensity

Parasite specie	No. Examined	No.(%) infected	(Mean±SD) Infection
<i>Ascaris suum</i>	300	112(37.3) ^a	22.4±16.2
<i>Ancylostoma spp.</i>	300	70(23.3) ^b	14.0±8.4
<i>Trichuris spp.</i>	300	48(16.0) ^c	9.6±7.6
<i>Trichinella spp.</i>	300	16(5.0) ^d	3.2±3.8
<i>Coccidial oocyst</i>	300	6(2.0) ^e	1.2±1.3

Different superscripts in columns differs significantly. $P < 0.00$

The distribution of the recovered parasite species and the effect of some epidemiological parameters (zootechnic category, health status and recent history of deworming) on the distribution are presented in Table 5. Based on the zootechnic categories; sows, dams and winners were the most infected with *Ascaris suum* and *Ancylostoma spp* in that order, while piglets and boars had similar prevalence of *Ancylostoma spp*.

However, boars had 39.47% infection rate with *Ancylostoma spp*. Similarly, boars were the most infected with *Trichuris suis* and was followed by dams (20.0%), winners (14.28%), sow (6.06%) and piglets (3.61%). *Trichinella spp.* was only found among boars and sows having 13.15% and 13.63% respectively, while winners had 3.17% infection rate. *Coccidial oocyst* had an infection rates of (5.26%), (4.54) and (1.58%) among boars, sows and winners respectively.

Table 5: Distribution of the parasite species based on zootechnic category, health status and history of deworming of Pigs from southern Adamawa State, Nigeria

Parameter Zootechnic Category (n)	Parasite No.(%) infection				
	<i>A. suum</i>	<i>A. spp</i>	<i>T.suis</i>	<i>Tri.spp</i>	<i>C.oocyst</i>
Piglets (83)	11(13.3) ^a	7(8.4) ^a	3(3.6) ^a	0(00.0) ^a	0(00.0) ^a
Winners (63)	20 (31.7) ^b	11(17.5) ^b	9(14.3) ^b	2(3.2) ^b	1(1.6) ^b
Dams (50)	30 (60.00) ^c	9(18.0) ^c	10(20.0) ^c	0(00.0) ^a	0(00.0) ^a
Sow (66)	46(69.7) ^d	28(42.4) ^d	4(6.0) ^d	9(13.6) ^c	3(4.5) ^c
Boar (38)	5(13.2) ^a	15(39.5) ^d	22(57.9) ^e	5(13.2) ^c	2(5.3) ^d
Health Status (n)					
Apparently Healthy (285)	100(35.0) ^a	63(22.1) ^a	44(15.4) ^a	4(1.4) ^a	4(1.4) ^a
Sick(15)	12 (80.0) ^b	7(46.7) ^b	4(26.7) ^b	12(80.0) ^b	2(13.3) ^b
History of deworming (n)					
Recently dewormed (45)	15(33.3) ^a	4(8.9) ^a	0(00.0) ^a	0(00.0) ^a	0(00.0) ^a
Not dewormed (255)	97(38.0) ^b	66(25.9) ^b	48(18.8) ^b	16(6.3) ^b	6(2.4) ^b

Different superscripts in columns differs significantly. $P < 0.05$

On health status of the examined animals, more parasites were recovered among sick pigs than their corresponding apparently healthy pigs. Similarly, pigs that were recently dewormed showed reduced frequency of gastrointestinal parasites compared to the non-dewormed. Parasites such as *Trichuris suis*, *Trichinella spp* and Coccidian oocyst were completely absent from the dewormed pigs.

Discussion

There have been previous attempts at evaluating the prevalence of gastrointestinal parasites in pigs under different husbandry systems in Nigeria (Salifu *et al.*, 1990; Sowemimo *et al.*, 2012; Pam *et al.*, 2013) and beyond (Weng *et al.*, 2005; Tomass *et al.*, 2013). Most of the studies reported varied prevalence. Despite the availability of well-documented evidence on the epidemiology of gastrointestinal parasitism in the North central and southern parts of Nigeria, there is little or no report to support this from the Northeastern part of Nigeria, ostensibly due to ethno-religious and cultural factors limiting the rearing and consumption of pigs to the relatively smaller Christian population in the region.

We report an overall prevalence rate of 84.0% in this study. The least infection of 69.2% was encountered among pigs from Mayo

Belwa, while the highest prevalence of 90.0% was among pigs from Numan local Government area. The higher infection rate in this study is consistent with the previous report by Weka and Ikeh (2009), who investigated intestinal parasitism among pigs in Jos, North central Nigeria. However, this contrasts earlier findings of Sowemimo *et al.* (2012) in Ibadan and Pam *et al.* (2013) in Jos, Nigeria, who reported lower rates of infection, probably due to the management system (intensive) under which the pigs were domesticated. Reports by Nsoso *et al.* (2000) and Kumsa and Kifle (2014) from African countries of Botswana and Ethiopia respectively showed lower infection rate compared with the present study. The high prevalence in this study may be due to the period of sampling and the type of production system. The type of management system has been shown to affect the prevalence of infection (Nansen and Roepstorff, 1999). Infective forms of parasites are known to be abundant in the environment and more easily available to their hosts during rainy season and under extensive and semi-intensive systems of management, where animals are allowed to scavenge. This may be the reason for the relative high infection rate. On species of parasite recovered in this study, the finding is similar to other workers from Nigeria (Salifu *et al.*, 1990; Weka and Ikeh, 2009) and beyond (Tamboura *et al.*, 2006;

Tomass *et al.*, 2013).

Parasites recovered in this study belong to the phylum Nematelminthes and Protozoa. This distribution may be attributed to the availability of infective forms in the environment and the technique used in recovery. The species of parasite recovered during the study are consistent with previous investigation in Nigeria (Tidi *et al.*, 2011) and other parts of the world (Tiwari *et al.*, 2009; Nsoso *et al.* 2000; Kumsa and Kifle, 2004). The frequent recovery of *Ascaris suum* accounting for 37% in this study further corroborates earlier study in Jos, Plateau state by Tidi *et al.* (2011), but contradicts results of similar study in southwestern, Nigeria (Sowemimo *et al.*, 2012). Parasites such as *Hyostromylus*, *Oesophagostomum* and *Globocephalus* reported previously (Tamboura *et al.*, 2006; Dey *et al.*, 2014) were not recovered in this study, and may be attributed to the unavailability of the infective forms of these parasites during the time of investigation. The technique used in the current study may be limited in its efficacy to detect other parasites especially of the class trematoda.

The effect of age and sex on the distribution of the parasite was investigated in this study, where age and sex were shown to significantly affect the distribution of the parasite. This finding contrasts earlier report of Kumsa and Kifle (2014) where these factors did not influence the prevalence of gastrointestinal infection. However, this is in agreement with previous workers (Nsoso *et al.*, 2000; Dey *et al.*, 2014). The high infection rate among the adult pigs may be due to relative long period of accessibility to the environment compared to the young pigs, coupled with risky behaviors such as feeding on garbage and contact with dirt which in turn increases exposure to infective form of the parasite. Similarly, female pigs studied had a significant infection rate than their corresponding males. Females generally are kept for longer period of time since they are required for reproduction, hence the longer period of exposure to infective forms of parasites. This however, is not the case with males that are normally sold out for meat on reaching market weight.

The zootechnic category of the pig, health status and recent history of deworming were among epidemiological factors whose effects were investigated on the prevalence of parasite. The recovery of more parasite species among dams, sows and boars compared with winners and piglets, is in agreement with the previous study by Dey *et al.* (2014) where adults and growers were more significantly ($p < 0.05$) affected than the piglets. This may be explained by the long period of stay which increases higher risky behaviors and access to infective forms of parasites as earlier observed. Similarly, in this study, animals that were observed to be apparently sick were more infected than the apparently healthy pigs, and may be explained by the fact that sick animals are prone to infections like parasitic infections, if not already infected. This may be due to lowered immune system. Similar observation was made among pigs that had no history of recent deworming, where there was a lower recovery rate of parasites among those that were recently dewormed.

This study has demonstrated the prevalence of gastrointestinal parasites consisting of mainly nematodes, which is consistent with previous studies in Nigeria and other parts of world. The findings provide baseline information on the prevalence and type of gastrointestinal parasites in pigs reared in southern part of Adamawa State, since no previous documented evidence exists on parasite prevalence from the region. Epidemiological factors such as age, sex, zootechnic category were all shown to determine the distribution of the parasites in pig.

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CHANGES IN BLOOD GLUCOSE AND PLASMA LIPIDS DURING GESTATION IN CHINCHILLA RABBITS SYNCHRONIZED WITH PREGNANT MARE SERUM GONADOTROPINS

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Abstract

Ten female Chinchilla rabbits with mean weight (1.9 ± 0.1 kg) were randomly assigned into two groups comprising of five each, to evaluate the changes in blood glucose and lipid profile during pregnancy. Control Group A was mated without prior synchronization, while rabbits in group B were synchronized with 0.15 mg/kg intramuscular injection of PMSG. Three days later, the group B rabbit does were mated naturally and confirmed pregnant seven days post mating using portable ultrasound machine. 5mls of blood was collected from the jugular vein before mating and 7, 14, 21 and 27 days post mating for the determination of blood glucose (GLU), total plasma cholesterol (tChol), triglycerides (TRIG), high density lipoproteins (HDL) and low density lipoproteins (LDL). The data were presented as mean \pm standard deviation and compared using analysis of variance for repeated measures. The results of this study showed that GLU decreased significantly ($P < 0.05$) by day 7 and 14 post mating in the control and PMSG synchronized rabbits respectively. Conversely, tChol increased by day 7 post mating and thereafter decreased significantly ($P < 0.05$) up to day 27 post mating. Both TRIG and LDL were significantly ($P < 0.05$) higher in control rabbits than the PMSG synchronized rabbits on day 7 post mating. However, there were no significant differences in HDL between the two groups of rabbits. In the PMSG synchronized rabbits, the HDL increased up to day 21 post mating and thereafter decreased by day 27 post mating, while in the control rabbit HDL increased up to day 14 post mating and then decreased by day 27 post mating. It was thus concluded that there were significant changes in the blood glucose and lipid profile during different stages of gestation in rabbits and that synchronization with PMSG influenced these changes.

Keywords: post-mating, triglycerides, lipoproteins, cholesterol

MODIFICATIONS DE LA GLYCEMIE SANGUINE ET DES LIPIDES PLASMATIQUES CHEZ LES LAPINES CHINCHILLA GRAVIDES SYNCHRONISEES AVEC DES GONADOTROPHINES SERIQUES DE JUMENTS GRAVIDES

Résumé

Dix lapines Chinchilla de poids moyen ($1,9 \pm 0,1$ kg) ont été réparties de manière aléatoire en deux groupes comprenant chacun cinq lapines, dans le but d'évaluer les modifications de la glycémie et du profil lipidique durant la gestation. Les lapines du Groupe témoin A ont été accouplées sans synchronisation œstrale préalable, tandis que les lapines du Groupe B ont été soumises à une synchronisation œstrale avec une injection intramusculaire de 0,15 mg / kg de PMSG. Trois jours plus tard, les lapines du Groupe B ont été accouplées naturellement, et ont confirmées gravides sept jours après l'accouplement en utilisant la machine à ultrasons portable. Cinq (5) ml de sang ont été prélevés à la veine jugulaire avant l'accouplement et à 7, 14, 21 et 27 jours après l'accouplement en vue de déterminer les taux de glycémie (GLU), de cholestérol total plasmatique (tChol), des triglycérides (TRIG), des lipoprotéines de haute densité (HDL) et des lipoprotéines de basse densité (LDL). Les données ont été présentées comme moyenne \pm écart-type et comparées en utilisant une analyse de variance pour les mesures répétées. Les résultats de cette étude ont montré que le taux de GLU a diminué de manière significative ($P < 0,05$) aux jours 7 et 14 après l'accouplement respectivement chez les lapines du Groupe témoin et celles synchronisées avec PMSG. Inversement, le taux de tChol a augmenté au jour 7 post-accouplement et a ensuite diminué de manière significative (P

<0,05) jusqu'au jour 27 post-accouplement. Les taux de TRIG et de LDL étaient significativement ($P < 0,05$) plus élevés chez les lapines du Groupe témoin par rapport aux lapines synchronisées avec PMSG au jour 7 post-accouplement. Cependant, on n'a pas noté de différences significatives au niveau des taux de HDL entre les deux groupes de lapines. Chez les lapines synchronisées avec PMSG, le taux de HDL a augmenté jusqu'au jour 21 post-accouplement et a par la suite diminué au jour 27 post-accouplement, tandis que la HDL des lapines du Groupe témoin a augmenté jusqu'au jour 14 après l'accouplement, puis a diminué au jour 27 après l'accouplement. L'étude a donc conclu qu'il y a eu des modifications importantes au niveau de la glycémie et du profil lipidique au cours des différentes étapes de la gestation des lapines et que la synchronisation œstrale avec PMSG avait influencé ces modifications.

Mots-clés : post-accouplement, triglycérides, lipoprotéines, cholestérol

Introduction

Pregnant animals experience physiological changes to support foetal growth and development. Among these is alteration in lipid and glucose metabolism with attendant adverse consequences (Mazur *et al.*, 2009). For instance, it has been reported in humans that increase in lipid and lipoprotein metabolism reaches a level of cardiovascular risk during the second trimester (Lippi *et al.*, 2007). In the ewe, increase in nutrient requirements at the third trimester of pregnancy have been shown to induce metabolic imbalances such as hypoglycemia and increase in the plasma concentration of free fatty acids (Piccione *et al.*, 2009). There appears to be contradictory reports on the changes that occur during pregnancy in different species of animals (Mizoguchi *et al.*, 2010), while very little is known about the changes that occur during pregnancy in the rabbits most especially the Chinchilla breed.

Pregnant rabbits are frequently and widely used in embryo-fetal development toxicity studies (Wells *et al.*, 1999). Interpretation of data from toxicology studies using pregnant animals may be difficult without the baseline data from the same breed or specie. However, very little is known about the metabolic changes that occur during pregnancy in rabbits. This study evaluated the changes in plasma concentration of glucose, cholesterol, triglycerides and lipoproteins during different stages of gestation in Chinchilla rabbits.

Materials and Methods

Animals

Ten primiparous female Chinchilla rabbits and four sexually matured chinchilla male rabbits with mean weight of 1.9 ± 0.1 kg were used. They were purchased from breeders located within Abeokuta metropolis and housed singly in wooden cages. The animals were fed with pelleted grower ration and forages (*Tridax procumbens*), while water was provided ad-libitum. They were maintained on twelve hours of day light and darkness cycle. Prior to commencement of the study, they were dewormed with Albendazole syrup at 22 mg/kg. All the rabbits were adjudged to be in good health based on the result of complete blood counts and physical examination. The protocol for this study was approved by the Ethics and Animal Welfare Committee of the Federal University of Agriculture, Abeokuta.

Design

A simple randomized design was used. Ten primiparous female Chinchilla rabbits were randomly assigned into two groups. The control group A comprised of five rabbits that were not synchronized prior to mating while the second group was synchronized with 20 IU of intramuscular injection of pregnant mare serum gonadotropins prior to mating (group B).

Experimental procedure

The rabbits were weighed and thereafter, rabbits in group B were synchronized with 20 IU of PMSG (Upjohn Pharmaceutical Limited, Crawley, Sussex, UK). Three days after, all the rabbit does were mated naturally four

times each using proven male rabbits so as to ensure that all the rabbit does were pregnant. Seven days after mating, the rabbits were confirmed pregnant using a portable ultrasound machine with a 10.0 MHz transducer (Kaixin KX 2000R, Xuzhou, China).

Blood Sampling

Five millimeter of blood was obtained from the jugular vein before mating and at 7, 14, 21 and 27 days post mating respectively for the determination of blood glucose and plasma lipid profile. Total plasma cholesterol, triglycerides and high density lipoproteins (HDL) and low density lipoproteins (LDL) were determined using Randox Laboratory kit reagents as described by Akpanabiatu *et al.*, 2005. The assay works on the principle of fluorometry. Cholesterol was measured enzymatically in plasma in a series of coupled reactions that hydrolyze cholesterol esters and oxidize the 3-OH group of cholesterol. The by product (H₂O₂) was then measured quantitatively in a peroxidase catalyzed reaction that produces a colour change. The absorbance of the colour intensity which is proportional to the plasma cholesterol concentration was measured at 500nm. Plasma triglycerides were measured enzymatically in a series of coupled reactions in

which triglycerides were hydrolyzed to produce glycerol. The glycerol was then oxidized into hydrogen peroxide whose absorbance was then measured at 500nm. Blood glucose was determined using automated blood analyzer (Beckman Coulter, UK).

Data analysis

Descriptive statistics such as mean and standard deviation were used to describe the changes that occurred in the blood glucose and plasma lipid measurements. The data was compared between the control and the synchronized rabbits using paired t test and analysis of variance for repeated measures. All statistical analysis was performed using statistical package for social sciences (SPSS) version 20.0.

Results

The blood glucose decreased significantly ($p < 0.05$) in control rabbits by day 7 post mating and the decrease were maintained up to day 21 post mating. Similarly, the blood glucose decreased significantly ($p < 0.05$) in PMSG treated rabbits up to 14 day post mating (Table 1). There was no significant difference ($p > 0.05$) in the blood glucose

Table 1: Changes in blood Glucose of Chinchilla rabbits during gestation

GROUPS	BM	7DPM	14DPM	21DPM	27DPM
CONTROL	97.5 ± 22.7 ^a	89.9 ± 7.5 ^b	70.8 ± 14.1 ^b	81.5 ± 13.2 ^b	88.3 ± 14.4 ^b
PMSG	110.0 ± 24.1 ^c	83.1 ± 11.6 ^a	70.2 ± 14.7 ^a	104.2 ± 15 ^a	102.0 ± 12.9 ^c

KEYS:

BM: Before mating ; 7DPM: 7 day post mating ; 14DPM: 14 day post mating ; 21DPM: 21 day post mating; 27DPM: 27 day post mating

$P \leq 0.05$ - Values with different superscript are statistically significant

Table 2: Changes in plasma total cholesterol of Chinchilla rabbits during gestation

GROUPS	BM	7DPM	14DPM	21DPM	27DPM
CONTROL	98.5 ± 13.9	121.4 ± 16.9 ^b	158.3 ± 27.3 ^c	100.0 ± 8.9 ^a	100.1 ± 19.7 ^a
PMSG	94.0 ± 14.7 ^a	120.1 ± 29.0 ^b	110.3 ± 28.7 ^a	105.3 ± 23.7 ^a	85.4 ± 13.0 ^a

KEYS:

BM: Before mating

7DPM: 7 day post mating

14DPM: 14 day post mating

21DPM: 21 day post mating

27DPM: 27 day post mating

$P \leq 0.05$

Values with different superscript are statistically significant

Table 3: Changes in plasma triglycerides of Chinchilla rabbits during gestation

GROUPS	BM	7DPM	14DPM	21DPM	27DPM
CONTROL	62.8 ±12.1 ^a	80.4 ±13.4 ^b	94.0 ±19.9 ^b	67.4 ±7.6 ^a	67.6 ±16.0 ^a
PMSG	63.6 ±10.7 ^a	80.5 ±20.5 ^b	67.9 ±13.0 ^a	71.3 ±20.9 ^a	56.5 ±9.1 ^a

KEYS:

BM: Before mating

7DPM: 7 day post mating

14DPM: 14 day post mating

21DPM: 21 day post mating

27DPM: 27 day post mating

P ≤ 0.05

Values with different superscript are statistically significant

Table 4: Changes in plasma high density lipoproteins (HDL) of Chinchilla rabbits during gestation

GROUPS	BM	7DPM	14DPM	21DPM	27DPM
CONTROL	22.4 ±7.8	24.5 ±7.7	35.8 ±11.0	30.4 ±8.5	24.0 ±8.8
PMSG	19.9 ±3.2	28.1 ±14.6	28.0 ±10.0	33.1 ±10.2	22.3 ±3.0

KEYS:

BM: Before mating

7DPM: 7 day post mating

14DPM: 14 day post mating

21DPM: 21 day post mating

27DPM: 27 day post mating

P ≤ 0.05

Values with different superscript are statistically significant

Table 5: Changes in plasma low density lipoproteins (LDL) of Chinchilla rabbits during gestation

GROUPS	BM	7DPM	14DPM	21DPM	27DPM
CONTROL	60.9 ±11.6 ^a	80.8 ±25.5 ^b	103.7 ±21.5 ^c	56.1 ±6.8 ^a	62.5 ±10.9 ^a
PMSG	61.5 ±12.3 ^a	75.9 ±31.7 ^b	68.8 ±19.2 ^a	58.0 ±12.5 ^a	53.1 ±15.9 ^a

KEYS:

BM: Before mating

7DPM: 7 day post mating

14DPM: 14 day post mating

21DPM: 21 day post mating

27DPM: 27 day post mating

P ≤ 0.05

Values with different superscript are statistically significant

between the PMSG and control rabbits except at 21 and 27 days post mating when the blood glucose was significantly ($p < 0.05$) higher in PMSG synchronized rabbits than the control rabbits. Conversely, the plasma cholesterol and triglycerides increased significantly ($p < 0.05$) by 7 days post mating in the two groups of rabbits (Table 2 & 3). However, there was no significant difference in the plasma cholesterol and triglycerides between the control and PMSG treated of rabbits throughout the duration of gestation except at day 14 post mating when the plasma cholesterol was significantly higher ($p < 0.05$) in the control rabbits than

the PMSG treated rabbits. The low density lipoprotein increased significantly ($p < 0.05$) by day 7 and day 14 post mating in the control rabbits while it increased by day 7 post mating in the PMSG treated rabbits (Table 4). The low density lipoprotein was only significantly ($p < 0.05$) higher in the control rabbits than PMSG rabbits on day 14 post mating. However, there were no significant changes in the high density lipoprotein throughout the duration of gestation in the rabbits (Table 5).

Discussion

The result of the study showed that blood glucose concentration decreased significantly up to fourteen day of gestation in the rabbits and thereafter increased progressively until the end of gestation. Conversely, both the total plasma cholesterol and triglycerides increased significantly up to fourteen day of gestation and thereafter decreased progressively until the end of pregnancy. These findings suggest that changes in the metabolism of glucose and carbohydrate occur during different stages of gestation in rabbits as seen in humans and other domestic animals.

Studies in human showed that glucose production increased throughout the course of pregnancy (Ekhatior and Ebomoyi, 2012). In ewes, glucose concentration decreased significantly in late gestation resulting in utilization of stored lipids for energy production, a condition known as pregnancy toxemia (Mazur *et al.*, 2009). Contrary to the observation in sheep, the result of this study showed a decrease in blood glucose concentration in the first two weeks of gestation and thereafter, a rise in the blood glucose concentration till the end of pregnancy. The decrease in blood glucose concentration in the first two weeks of gestation in the rabbit might be due to increase in glucose demand for early embryonal development. It may be that adequate feeding of the rabbit with concentrates ensured sufficient production of glucose, hence the increase in the blood glucose concentration towards the late gestation period.

Maternal cholesterol is an important source of cholesterol for the fetus during early gestation (Diareme *et al.*, 2009). Its importance becomes minimal in late pregnancy due to the high capacity of the fetal tissues to synthesize cholesterol. This might explain the changes observed in the total plasma cholesterol concentration of the rabbit does. At the first two weeks of gestation, the total plasma cholesterol increased due to lipogenesis and fat storage in preparation for rapid fetal growth in late pregnancy (Ekhatior and Ebomoyi, 2012). The decrease in plasma concentration of

cholesterol in the rabbit during the last two weeks of gestation might be due to a decrease in the hepatic lipase activity. Hepatic lipase is responsible for the increased synthesis of triglycerides at the hepatic level (Patrizia *et al.*, 1999). It may also be due to decrease in production of maternal cholesterol since the fetal is able to synthesize cholesterol at late gestation (Diareme *et al.*, 2009).

It has been reported that during late gestation in pregnant ewe on adequate diet, the plasma triglyceride concentration increased significantly (Mazur *et al.*, 2009). In this study, the plasma triglycerides of the rabbits at late gestation did not differ from the values obtained before mating. This showed that there is no significant alteration in triglyceride metabolism during late gestation in the rabbit. Only the low density lipoprotein fraction differs significantly during gestation in the rabbits. The increase in the plasma low density lipoprotein coincides with the period of increase in the plasma triglyceride concentrations in the rabbits. This suggested that there was an increase synthesis of low density lipoprotein fraction in order to mobilize triglycerides from the plasma into the liver (Diareme *et al.*, 2009).

Finally, synchronization with PMSG appeared to have significantly altered the blood glucose in in this study suggesting that synchronizing the rabbits prior to mating significantly influence both the litter size, as well as increasing the receptivity of the rabbit does to the males. It may also be that the presence of the male rabbits might have primed the does that were not synchronized and thus improving receptivity.

In conclusion, the result of this study showed that blood glucose concentration is increased during late gestation in rabbit contrary to that observed in sheep. However, the plasma concentration of cholesterol, triglycerides and high density lipoprotein decreased during late gestation in rabbits probably due to the increase synthesis of cholesterol by fetal tissues towards late gestation.

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CONTROLE DE LA TRYPANOSOMOSE BOVINE ET DE SES VECTEURS PAR L'UTILISATION DE LA CYPERMETHRINE 3% EN POUR-ON DANS LA ZONE INFESTEE DE L'ADAMAOUA, CAMEROUN.

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Résumé

L'étude a été menée à Almé de Décembre 2013 à Avril 2014. Elle avait pour objet de tester l'efficacité de la cyperméthrine 3% high-cis en "pour-on" (Didétéki®) comme moyen de contrôle de la trypanosomose bovine et de ses vecteurs. 209 bovins ont été échantillonnés pour évaluer la prévalence de la trypanosomose bovine. Les moyens de diagnostic utilisés ont été la technique du buffy coat et le frottis sanguin coloré au Giemsa. La prévalence parasitologique observée a été de 29,18% et trois espèces de trypanosomes sont identifiées dont *T. vivax* (49,19 %), *T. congolense* (26,22%) et *T. brucei* (24,59 %). L'hématocrite moyen était de 24,29±4,38 %. Deux troupeaux de mâles entiers de 2 et 3 ans ont été constitués et traités mensuellement à la cyperméthrine 3% en "pour-on" à la dose de 1 mL/10 Kg de poids vif. Au terme de l'étude, la réduction de l'incidence de la trypanosomose bovine est de 85,76% avec une augmentation de l'hématocrite de 2,55 %. Les prospections entomologiques montrent une baisse de la densité apparente des vecteurs qui passe de 1,12 à 0,2 glossine/piège/jour.

Mots clés : Bovins, trypanosomose bovine, prévalence, hématocrite, contrôle, cyperméthrine, pour-on, vecteurs.

CONTROL OF BOVINE TRYPANOSOMOSIS AND ITS VECTORS THROUGH 3% CYPERMETHRIN POUR-ON, IN THE INFESTED AREA OF ADAMAOUA, CAMEROON.

Abstract

This study was conducted in Almé, from December 2013 to April 2014. Its aim was to assess how effective 3% cypermethrin high-cis "pour-on" (Didétéki®) was in the control of bovine trypanosomiasis and its vectors. 209 cattle were sampled to assess the prevalence of bovine trypanosomiasis. Diagnostic means used were the buffy coat and Giemsa-stained blood smear. The parasitological prevalence observed was 29.18%; and three trypanosome species were identified, including *T. vivax* (49.19%), *T. congolense* (26.22%) and *T. brucei* (24.59%). The mean packed cell volume was 24.29 ± 4.38%. Two herds of uncastrated males aged 2 and 3 years were formed and treated monthly with 3% cypermethrin "pour-on" in a dosage of 1 mL / 10 kg of live weight. At the end of the study, the reduction in bovine trypanosomiasis incidence was 85.76% with an increased packed cell volume of 2.55%. Entomological surveys show a decreased apparent density of vectors from 1.12 to 0.2 tsetse/trap/day.

Keywords: cattle: bovine trypanosomosis ; prevalence ; packed cell volume ; control ; cypermethrin ; pour-on ; vectors

Introduction

La région de l'Adamaoua est le plus grand bassin de production du bétail au Cameroun. Elle représente à elle seule 28 % du cheptel national qui est estimé à 10 millions de bovins (1). Conscient de ce potentiel et depuis l'invasion de l'Adamaoua en 1950 par les glossines (*Glossina morsitans submorsitans*, *G.tachinoides* et *G.fuscipes fuscipes*), des pulvérisations aériennes, terrestres et la pose d'écrans et pièges se sont succédés de 1976 à 1994 (2). Au terme de cette campagne, l'Adamaoua se scinde en trois zones : une zone infestée, une zone tampon et une zone assainie (3). Dans la région de l'Adamaoua, la trypanosomose bovine est la pathologie majeure donc les prévalences les plus élevées sont rencontrées dans la zone infestée caractérisée par la présence constante des glossines (4, 5), où aucune lutte antivectorielle n'a été menée. De surcroît, la résistance aux trypanocides dont le diminazène et l'isométhamidium, a été relevée (6) de même que des souches de trypanosomes résistants dans le Faro et Déo (7). Le ralentissement des activités de la mission spéciale d'éradication des glossines (MSEG créée en 1974) dus à la baisse du budget, poussent les éleveurs à s'investir eux-mêmes dans la lutte antivectorielle surtout ceux de la zone infestée. Compte tenu du système d'élevage, des feux de brousse et de l'absence de zones de pâturages bien délimitées, les pyrèthroides de synthèse en "pour-on", est le moyen de lutte le plus utilisé dans l'Adamaoua de nos jours (8). Bien que plusieurs études ont été menées dans plusieurs pays en Afrique sur ces formulations d'insecticides et ont prouvés leur efficacité quant à la lutte contre la trypanosomose bovine et ses vecteurs (9, 10, 11), aucune étude n'a été encore menée au Cameroun sur ce produit pourtant largement utilisée. Ainsi donc 146 animaux de 2 et 3 ans ont été traités mensuellement à la cyperméthrine 3% high-cis en "pour-on" (Didétéki®). Cette étude a été complétée par des prospections entomologiques de cinq mois pour évaluer la diversité et la densité de l'entomofaune piqueuse pendant le traitement.

Matériel et méthodes

Zone d'étude

L'étude a été menée de Décembre 2013 à Avril 2014 dans le ranch Nana Bouba à Almé précisément dans le village Sarkimata. Le département du Faro et Déo a une superficie de près de 11.000 Km² et s'étend du 7ème au 8ème degré de latitude Nord et du 12ème au 13ème degré de longitude Est (figure 1). Il est limité au nord par la réserve du Faro et le département du Faro (région du Nord), au sud par le département de Mayo Banyo, à l'est par le département de la Vina et à l'ouest par la frontière nigériane (dont la réserve forestière de Gashaka). La plaine Koutine et précisément Almé, est une zone de transhumance par excellence et une zone importante d'élevage des bovins car elle représente à elle seul environ 10% du cheptel bovin du département du Faro et Déo qui est estimé à 74.559 têtes (5). La pathologie la plus rencontrée est la trypanosomose bovine. Les races bovines rencontrées sont les zébus Goudali, White fulani, Red fulani et Brahmane. On y retrouve une population composé de Koutine (majoritairement), Peuls et Mbororo dont les activités principales sont l'agriculture (céréales, tubercules), l'élevage (bovins, ovins) et le commerce. Les espèces de glossines qu'on retrouve sont essentiellement au nombre de deux: *Glossina morsitans submorsitans* et *G.tachinoides* (4).

La végétation de type savane est dense et constituée de *Daniellia oliveria*, *Lophira lanceolata* et *Isoberlinia doka*. La strate herbacée à *Hyparrhenia* sp., *Panicum* sp. et *Sporobolus africanus*, est constamment en évolution sous l'action des facteurs anthropiques (pâturage, feux de brousse, défrichage) édaphiques et ou climatiques (6,3). Le climat est soudano-sahélien et caractérisé par 2 saisons : une saison sèche (novembre à mars) et une saison pluvieuse (avril à octobre). Les pluviométries en 2012 et 2013 sont respectivement de 1531,3 mm et 1195,7mm d'eau. La température moyenne annuelle est de 28°C (avec un maximum de 40°C) (6).

Prélèvement de sang et analyse parasitologique

Avant le traitement et afin de connaître le risque trypanosomien, la prévalence de la trypanosomose bovine et l'hématocrite moyen ont été déterminés. Ainsi donc, 209 bovins mâles (149) et femelles (60), âgés de 1 à 7 ans, de races zébus Goudali, White fulani, Red fulani et Brahmane ont été échantillonnés aléatoirement en décembre 2013 dans le but de choisir deux troupeaux présentant les prévalences les plus élevées avec des hématocrites moyens les plus faibles. Le périmètre thoracique mesuré à l'aide d'un ruban barymétrique a permis d'évaluer le poids vif selon la formule établie par Njoya *et al.* (12). Celle-ci est adaptée pour les zébus du grand Nord-Cameroun, mâle et femelle de plus de 50 Kg :

- Pour les mâles: Poids (kg) = $100,264 - 2,641 \times PTHO + 0,0251 \times PTHO^2$ ($R^2 = 0,96$)
- Pour les femelles: Poids (kg) = $124,69 - 3,171 \times PTHO + 0,0276 \times PTHO^2$ ($R^2 = 0,96$)

PTHO = périmètre thoracique.

La méthode de diagnostic parasitologique utilisée est la technique du buffy coat. Environ 3 mL de sang sont prélevés de la veine jugulaire dans des tubes de prélèvement

de 5 mL (Vacutest®) avec anticoagulant (EDTA). Chaque échantillon de sang est aspiré par capillarité dans un tube à microhématocrite et centrifugés à 12000 tours/min (Hawksley & sons Limited®, UK) pendant 5 minutes. L'hématocrite est déterminé grâce au lecteur à hématocrite (Hawksley Microhematocrit Reader®). Des frottis sanguins des échantillons positifs au buffy coat sont colorés au Giemsa dilué, afin de poser la diagnose des espèces de trypanosomes (13). Le degré de parasitémie a été déterminé selon l'échelle (de +1 à +6) établie par Paris *et al.* (14). Les animaux positifs ont été traités avec du diminazène (Vériben B12®) à la dose de 7 mg/Kg de poids vif.

Le dernier traitement trypanocide et la vermifugation des animaux échantillonnés, datent respectivement de septembre et mai 2013. Les animaux ont été vaccinés contre les grandes épizooties notamment la péripneumonie contagieuse bovine, le charbon symptomatique, la maladie nodulaire et la pasteurellose. Pendant toute l'année et à cause de la proximité avec la réserve du Faro, des babouins, des antilopes, des phacochères etc. sont fréquemment rencontrés dans le ranch.



Figure 1 : Site de l'étude (département du Faro et deo)

Application de la cyperméthrine 3% en "pour-on"

Les deux troupeaux les plus infestées et présentant un hématoците faible, ont été retenus pour cette étude. Le troupeau 1 (72 animaux) et le troupeau 2 (74 animaux) sont constitués des mâles entiers âgés respectivement de 2 et 3 ans. Le troupeau 1 et 2 sont composés de zébus Goudali, Brahmane, Red fulani et White fulani dont les proportions respectives sont: 75% et 90,54% ; 2,77% et 2,70% ; 19,46% et 5,40% ; 2,77% et 1,33%.

Avant le traitement à la cyperméthrine 3% en "pour-on", les deux troupeaux ont été traités systématiquement avec du diminazène (Vériben B12®, 7 mg/Kg de poids vif) pour «effacer» les infections trypanosomiennes et éliminer l'influence des autres pathologies avec de l'oxytétracycline (Wolicyclin 20% L.A.®, 20 mg/Kg de poids vif) et l'albendazole (Afridazole KE LA®, 10mg/Kg de poids vif).

La cyperméthrine 3 % en "pour-on" (Didétéki® ; N° du lot : 11L08.10) a été administré aux deux lots, mensuellement (à la dose de 1 mL/10 Kg de poids vif) de janvier à avril 2014. Le médicament est déposé le long de la colonne vertébrale c'est-à-dire de la base du cou à la base de la queue. Trente animaux (30) par troupeau sont échantillonnés aléatoirement toutes les deux semaines pendant 3 mois afin de suivre l'évolution de l'incidence de la trypanosomose bovine et de l'hématoците. L'intervalle de deux semaines correspond à la moyenne de la période d'incubation de la trypanosomose bovine qui est de 8-20 jours (15). A cause de la rémanence du diminazène qui est de 3 semaines (16), le premier prélèvement du suivi (4ème semaine) a lieu un mois (S4) après l'administration du Vériben B12®. Les animaux positifs sont traités au diminazène et sont écartés du suivi.

Prospections entomologiques et identification des glossines

Dans le but de déterminer les vecteurs de la trypanosomose et leurs densités, des prospections entomologiques ont été réalisées de décembre 2013 à avril 2014. Pour ce faire, 5 pièges biconiques Challier-Laveissière appâtés avec de l'acétone (appât olfactif) contenu dans un flacon et disposé au pied de chaque piège

diffusant à un débit de 500 mg/h (17) ont été utilisés. Ils sont fixés à 30 cm du sol et espacés entre eux de 200 mètres environ. L'emplacement des pièges est dégagé, ensoleillé, visible, près du bord de l'eau (17). Ces prospections, menées en 5 jours consécutifs par mois (18) ont été réalisées dans trois sites dont à l'entrée du ranch et près des campements et dans deux zones de pâturages.

La densité apparente des glossines par piège (DAP) et par jour a été évaluée mensuellement selon la formule (19) :

DAP= Nombre total de glossines/ (nombre total de pièges x nombre de jours de capture)

Les insectes vecteurs capturés (vecteurs mécaniques et glossines), ont été identifiés à l'institut de recherche agricole pour le développement (IRAD), station de Wakwa Ngaoundéré. Les critères morphologiques d'identification des glossines sont les formes, les dimensions, les caractéristiques des ailes (cellule en hache, positionnement des ailes), l'aspect de la genitalia (pour différencier les mâles des femelles), la couleur des articles tarsaux, etc. (20).

Analyse des données

Les données obtenues ont été analysées avec le logiciel SPSS (Statistical Package for the Social Sciences) version 20.0. Les animaux ont été répartis par classe d'âge (0-1 an, 2-3 ans et 4-7 ans) telle définis par Kamuanga et Kabore (21) et par sexe (mâle et femelle). Le taux d'hématoците a été défini en deux catégories (anémie ($\leq 24\%$) et normale ($> 24\%$)) (22). Les tests de Khi deux de Pearson ou ANOVA ont été utilisés pour comparer les proportions ou les moyennes au seuil de signification de 5%.

Résultats

Prévalence parasitologique de la trypanosomose et hématoците moyen des animaux examinés

Des 209 échantillons de sang analysés, 61 animaux sont détectés positifs à la trypanosomose, soit une prévalence de 29,18%. Trois espèces de trypanosomes ont

été identifiées à des proportions différentes : *Trypanosoma vivax* (49,19%), *T. congolense* (26,22%) et *T. brucei* (24,59%). Il n'y a pas d'infection mixte. La parasitémie la plus rencontrée est de 103 -104 et 5×10^4 à 5×10^5 trypanosomes/mL. Le tableau 1 montre un taux d'infection élevé chez les femelles et dans la tranche d'âge 5-7 ans : Les analyses statistiques montrent que, la prévalence ne diffère pas significativement entre les classes d'âge ($p=0,574$), et le sexe ($p=0,402$).

L'hématocrite moyen des animaux examinés est de $24,29 \pm 4,38$ % (tableau 2). Les vaches présentent un hématocrite moyen plus faible (23,48%) comparé aux taureaux. Les hématocrites moyens les plus basses sont enregistrés chez les individus infectés (21,21%). La différence est significative entre l'hématocrite moyen des individus malades et sains ($p=0,001$) et dans les classes d'âges ($p=0,001$).

Effets de la cyperméthrine 3% en "pour-on" sur l'incidence de la trypanosomose et l'hématocrite

Pendant l'application de la cyperméthrine 3%, l'incidence du troupeau 1 passe de 6,66% à la 4ème semaine (S4) après le traitement à 1,33% à la 10ème semaine (S10) et celle du troupeau 2 de 16,66% (S4) à 0% (S10). L'incidence de façon générale passe de 11,66%

(S4) et à 1,66 % (S8 et S10), soit une réduction de 85,76%. On note une différence significative ($p=0,038$) entre l'incidence de la S4 et celles des S8 et S10 (figure 2).

L'hématocrite par contre a augmenté de 2,55 % (de 29,13 % à 31,68 %) entre la S4 et la S10. Il y a une différence significative entre les semaines ($p=0,004$) et précisément entre la S4 et la S8 d'une part et d'autre part entre la S4 et la S10. Dans le troupeau 1 cette hausse est de 2,17% contre 2,93% dans le troupeau 2. La figure 2 illustre l'évolution de l'incidence et de l'hématocrite moyenne des deux troupeaux de la 4ème semaine à la 10ème semaine (de février à avril).

Prospections entomologiques

Au terme de cinq mois de capture, 67 glossines (72,82%) et 25 tabanidés (27,18%) ont été capturés soit une densité apparente moyenne de 0,54 glossine/piège/jour. La DAP en décembre, janvier, février, mars et avril sont respectivement de 0,04 ; 1,12 ; 0,84 ; 0,48 et 0,02. *Glossina morsitans submorsitans* (73,13%) est l'espèce de glossine prédominante comparée à *G. tachinoides* (26,87%). Plus de glossines mâles (73,14%) que de femelles (26,86%) ont été capturés. Il a été noté une corrélation positive entre la baisse de l'incidence et celle de la DAP telle qu'illustre la figure 3.

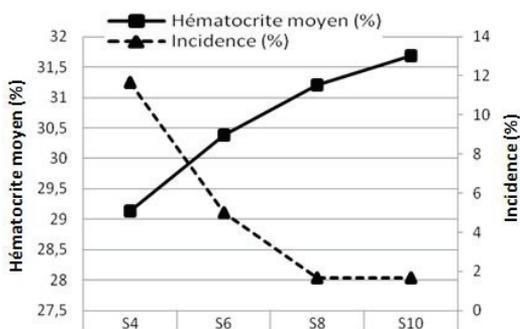


Figure 2: Evolution de l'incidence de la trypanosomose bovine et de l'hématocrite moyen S (Semaine)

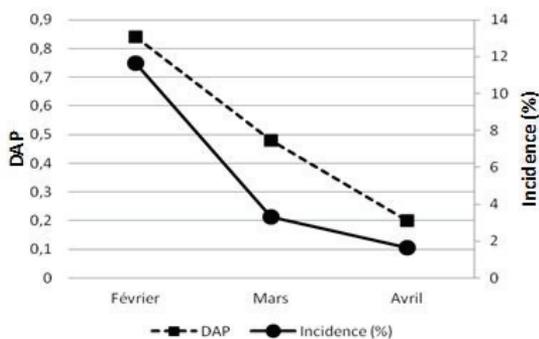


Figure 3 : Evolution de la densité apparente de glossine et de l'incidence de la trypanosomose bovine

Tableau 1 : Prévalence parasitologique de la trypanosomose bovine par tranche d'âge et par sexe

	Facteurs de variation	Nombre d'animaux examinés	Nombre d'animaux infectés	Prévalence (%)	Valeurs de p
Tranche d'âge	0-1 an	52	16	30,76	0,574
	2-3 ans	97	25	25,77	
	5-7 ans	60	20	33,33	
Sexe	Mâle	149	41	27,51	0,402
	Femelle	60	20	33,33	

Tableau 2 : Variation de l'hématocrite moyen en fonction du groupe d'âge, du sexe et du type d'infection

	Facteurs de variations	Hématocrite moyen (%)	Valeur de p
Classe d'âge	0-1 an	22,98±4,01	0,001
	2-3 ans	25,50± 4,29	
	5-7 ans	23,48± 4,39	
Sexe	Mâle	24,62±4,35	0,089
	Femelle	23,48±4,49	
Statut	Négatif	25,56±3,91	
	Positif	21,21±4,38	
Type d'infection	<i>T. congolense</i>	20,06±4,80	0,299
	<i>T. brucei</i>	22,26±2,91	
	<i>T. vivax</i>	21,30±3,85	

Hématocrite moyen = moyenne ± écart-type

Discussion

La prévalence parasitologique de la trypanosomose bovine dans cette présente étude est de 29,18%, plus élevée que celle obtenue par Mamoudou *et al.* (5) dans le Faro et Déo qui est de 23%. Ce résultat est élevé parce qu'Almé est situé dans la vallée (dans le département du Faro et Déo) qui est une zone infestée contrairement à la zone assainie du même département. De plus, il est situé entre deux réserves dont la réserve forestière de Gashaka (au nigéria) et la réserve du Faro (Cameroun) qui sont des réservoirs de glossines, et ceux-ci envahissent la plaine en saison sèche dès l'arrivée des troupeaux du plateau et du Nigeria venus passer la transhumance (5). Le risque d'infection est élevé en saison sèche (14, 23). De plus, les helminthoses gastro-intestinales rendent susceptibles les bovins à la trypanosomose.

T. vivax (49,19%) est prédominante comparé à *T. congolense* (26,22%) et *T. brucei* (24,59%) est due probablement aux tabanidés, vecteurs mécaniques par excellence de *T. vivax* et aussi des contacts répétés avec des glossines du groupe morsitans (20, 24). La complexité du cycle évolutif *T. brucei* chez la glossine justifie sa faible proportion (3). De plus, le taux d'infection des glossines, particulièrement du groupe morsitans à *T. vivax* est élevé (20%), faible pour *T. congolense* et très faible pour *T. brucei* (14, 20). La faible parasitémie constatée est probablement due au fait que la maladie évolue rapidement vers la chronicité. De telles proportions sont également rapportées par Sam-Wobo *et al.* (25) au Nigéria et par Mpouam *et al.* (24) dans l'Adamaoua au Cameroun.

Les bovins de 0-1 an pâturant près des campements où la DAP est faible (0,04), présentent une prévalence élevée (30,76%) comparée aux animaux de 5-7 ans qui sont

les infectés. Les vaches, à cause de leur état physiologique (allaitement, gestation...), se contentent des pâturages à proximité et chez qui les ectoparasites sont en grand nombre, présentent une prévalence élevée contrairement aux mâles où elle est faible car ils vont dans les pâturages protégés par les écrans bleus situés plus loin.

L'hématocrite moyen qui est de 24,29 %, témoigne d'un état d'anémie dans le cheptel du probablement à la trypanosomose dont la prévalence est élevée, témoignant ainsi du caractère anémiant de cette pathologie, constat fait également par Van Den Bossche et Rowlands (26). Sur les 45,93% des animaux présentant un hématocrite inférieur ou égal à 24%, 49,87% seulement sont atteints de trypanosomose. Ceci laisse entrevoir d'autres facteurs ou maladies anémiants tels que les helminthoses gastro-intestinales (27), les hémoparasitoses (outre que la trypanosomose), les ectoparasites (tiques) et les déficiences nutritionnelles (5, 28).

L'hématocrite moyen faible rencontré chez les bovins parasités par *T. congolense*, est due à sa pathogénicité élevée comparé aux autres trypanosomes (15). Tanenbe *et al.* (3) et Mpouam *et al.* (24) ont abouti à des conclusions similaires. De façon générale l'hématocrite moyen est influencé par l'âge, le niveau génétique (15) et la prévalence (26) et du statut parasitologique des animaux (les bovins parasités présentent un hématocrite moyen plus faibles que ceux qui sont sains).

Lors du traitement à la cyperméthrine 3% en "pour-on", de la S4 à la S10, 12 animaux au total ont été diagnostiqués positifs à la trypanosomose soit une incidence moyenne de 5 %. La réduction de l'incidence est 85,76% inférieure à celui obtenu par Okiria *et al.* (10) qui est de 92,30% et Van Den Bossche *et al.* (29) ne diagnostiquent aucune infection trypanosomiène au 10^{ème} mois du suivi. La cyperméthrine appliquée sur les animaux, éliminera non seulement les vecteurs (effets kill et knock down) et/ou limitera la pose (effets irritant et répulsif), par conséquent contribuera à la baisse de l'incidence de la trypanosomose bovine. D'ailleurs, au cours de l'étude, on a noté une corrélation positive

entre la baisse de la densité des vecteurs et l'incidence de la trypanosomose bovine.

L'albendazole aurait joué un rôle car l'administration d'un anthelminthique augmenterait l'immunité des animaux face à la trypanosomose (30). De plus, la baisse de la charge parasitaire notée pendant l'application de la cyperméthrine, constat fait également par Nonga *et al.* (31), améliorera la santé des animaux et accroîtra leur résistance à la trypanosomose (32).

Entre la S4 et la S10, l'incidence du troupeau 2 (de 6,66% à 0%) décroît plus rapidement comparé au troupeau 1 (de 16,66% à 3,33%). Ces résultats montrent que les animaux de 3 ans (troupeau 2) réagissent mieux à la cyperméthrine 3% comparés à ceux âgés de 2 ans (troupeau 1). Rowlands *et al.* (18) rapportent plutôt une réduction de l'incidence de 66% et 62% respectivement chez les bovins de 2 ans et 3 ans et plus.

Toutefois, la trypanosomose a persisté parce que l'effet répulsif et irritant des pyréthrénoïdes de synthèse ne peut que réduire sans empêcher totalement la pose, la prise du repas sanguin ou la transmission de la maladie (29). De plus, les membres antérieurs, particulièrement les parties basses, sont préférés par les glossines et spécialement *Glossina tachinoides* (33). Des études ont montré que la concentration d'insecticides persiste sur le dos contrairement aux membres où les concentrations sont faibles (32).

La cobalamine contenu dans le Vériben B12® aura corrigé le déséquilibre causé par la trypanosomose et favorisera la remontée rapide de l'hématocrite, d'où une augmentation de 9,05 % entre la S0 et la S4 (34, 35). De façon générale, la hausse de l'hématocrite moyen de 2,55% de la S4 à la S10, sera due à albendazole qui a éliminé les helminthes, spoliateur non négligeable (36) et aussi à la baisse de l'infection trypanosomiène dont l'incidence (18, 29).

L'hématocrite moyen du troupeau 2 a augmenté de 2,93 % plus que celui du troupeau 1 (2,17 %). Cette différence serait due à la composition raciale des troupeaux car le troupeau 2 est une grande proportion de zébus Goudali comparé au troupeau 1, mais par contre le troupeau 1 a une proportion plus

grande de zébu fulani. Or l'hématocrite des zébus Goudali est supérieur à celui des zébus fulani (37, 38). De plus, les animaux du troupeau 2, plus âgés, se rétablissent mieux de l'infection.

Toutefois, cette augmentation de l'hématocrite semble faible à cause de l'alimentation (fourrage sec) et de la rudesse de la saison sèche. D'ailleurs, les études menées par Sawadogo *et al.* (39) ont montré que certains paramètres biochimiques (protéines, glucose, hématocrite...) baissent chez les animaux nourris uniquement sur un pâturage pauvre et sec.

Glossina morsitans submorsitans est l'espèce prédominante comparée à G. tachinoides constat fait aussi par Mamoudou *et al.* (28). Ceci sera dû à l'écologie de ces vecteurs et à l'acétone, attractif efficace sur G. morsitans submorsitans comparé à G. tachinoides et aux tabanidés (17, 40). La proportion de femelles capturées est faible, soit 20,40 % et 44,44 % respectivement pour G. morsitans submorsitans et G. tachinoides. Mamoudou *et al.* (28) rapportent les mêmes résultats dans le Faro et Déo. Politzar et Merot (41) au Burkina-Faso, mentionnent une capture élevée de G. morsitans submorsitans mâle lorsque les pièges biconiques sont appâtés à l'acétone. La DAP faible à l'entrée de l'exploitation et près des campements mais élevée dans les pâturages, sera due à l'occupation de l'homme qui entraîne le recul des glossines. La DAP qui baisse progressivement de janvier (1, 12) à avril (0, 20), qui est la période de traitement. Plusieurs études ont montré que des traitements réguliers avec des pyréthrénoïdes de synthèse tels que la cyperméthrine, la deltaméthrine et la cyfluthrine, réduisent l'incidence de la trypanosomose bovine (42, 9) non seulement à cause de ses effets létaux et knock down, mais aussi grâce à ses effets répulsifs et irritants vis-à-vis des glossines et des vecteurs mécaniques par extension (32, 43, 44). De surcroît en saison sèche, l'effet de la cyperméthrine est marqué et rapide car il n'y a ni pluie, ni brossage par de hautes herbes (29). Les feux de brousse et la faible hygrométrie en saison sèche auraient également contribué à la baisse de la DAP (10, 45).

Des traitements réguliers des bovins de

la localité de la zone infestée à la cyperméthrine 3% en "pour-on", contribueront à abaisser la prévalence de la trypanosomose et à éliminer les vecteurs de cette maladie. L'effet du traitement sur les paramètres de reproduction et de productivité pourrait être évalué.

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EFFECTS OF AGE AND SEX ON HAEMATOLOGICAL AND SERUM BIOCHEMISTRY IN JAPANESE QUAILS

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Abstract

This study was conducted to determine the variations in blood parameters (haematology and serum biochemistry) at different ages (in weeks) in male and female Japanese quails. A total of hundred (100) unsexed day-old quail chicks were purchased and sorted based on sex at the third week. Blood samples were collected at the 3rd, 5th, 7th and 9th week of age from each sex and analysed for the haematological and serum components. The experiment was arranged in a 4 x 2 factorial layout and all data collected were subjected to one way analysis of variance in completely randomized design (CRD) using SPSS version 20 for windows. Haematological indices were not significantly ($P > 0.05$) influenced by age except ($P < 0.05$) haemoglobin which increased with increasing age. However, none of the haematological parameters were different ($P > 0.05$) between the sexes. Total protein, albumin and globulin decreased significantly ($P < 0.05$) with increasing age, however significant ($P < 0.05$) increase were noted for serum calcium, Aspartate transaminase (AST) and Alanine transaminase (ALT). Cholesterol was the only serum biochemical parameter that varied significantly ($P < 0.05$) between the sexes with the highest (131.88mg/d) shown in females. All determined haematological indices did not indicate any significant ($P > 0.5$) effect of interaction between age and sex in quails except ($P < 0.05$) for the PCV which was highest in male quails at the 9th week (51.00%) and least in male quails at the 3rd week (36.50%). All serum indices were statistically different ($P < 0.05$) except calcium and uric acid. Based on the results of this study, it can be concluded that haemoglobin increased with advancement in age while other haematological parameters were not affected. Sex did not influence haematological parameters in quails, however, female quails had higher serum cholesterol than male quails.

Keywords: age, sex, haematology, serum biochemistry and Japanese quails

EFFETS DE L'AGE ET DU SEXE SUR LA BIOCHIMIE HEMATOLOGIQUE ET SERIQUE DES CAILLES JAPONAISES

Résumé

Cette étude a été menée dans le but de déterminer les variations des paramètres sanguins (hématologie et biochimie sanguine) des cailles japonaises mâles et femelles à des âges différents (semaines). Au total, cent (100) poussins d'un jour non différenciés par sexe ont été achetés et triés sur la base du sexe à la troisième semaine. Des échantillons de sang ont été prélevés sur des poussins des deux sexes, aux 3ème, 5ème, 7ème et 9èmes semaines d'âge, et analysés pour déterminer les composants hématologiques et sériques. L'expérience a été organisée selon une configuration factorielle 4 x 2, et toutes les données recueillies ont été soumises à une seule analyse unidirectionnelle de variance dans un schéma complètement aléatoire (CRD), à l'aide du logiciel SPSS version 20 pour Windows. Les indices hématologiques n'ont pas été significativement ($P > 0,05$) influencés par l'âge, à l'exception de l'hémoglobine ($P < 0,05$) qui a augmenté avec l'âge. Cependant, on n'a pas noté de différence au niveau des paramètres hématologiques ($P > 0,05$) entre les sexes. Les protéines totales, l'albumine et la globuline ont diminué de manière significative ($P < 0,05$) avec l'augmentation de l'âge ; cependant, une augmentation significative ($P < 0,05$) a été observée pour le calcium sérique, l'aspartate transaminase (AST) et l'alanine transaminase (ALT). Le cholestérol est le seul paramètre biochimique sérique qui a varié de façon significative ($P < 0,05$) entre les deux sexes, le plus élevé (131,88mg/d) ayant été noté chez les femelles. Tous les indices hématologiques déterminés n'ont pas indiqué un quelconque effet significatif ($P > 0,5$) de l'interaction entre l'âge et le sexe des cailles, sauf (P

<0,05) pour le PCV qui était très élevé chez les cailles mâles à la 9^{ème} semaine (51,00%) et très bas chez les cailles mâles à la 3^{ème} semaine (36,50%). Tous les indices sériques étaient statistiquement différents ($P < 0,05$), à l'exception du calcium et de l'acide urique. Sur la base des résultats de cette étude, on peut conclure que l'hémoglobine a augmenté avec l'âge, alors que les autres paramètres hématologiques n'ont pas été affectés. Le sexe n'a pas influencé les paramètres hématologiques des cailles ; cependant, les cailles femelles avaient un taux de cholestérol sérique supérieur à celui des cailles mâles.

Mots-clés : âge ; sexe ; hématologie ; biochimie sérique et cailles japonaises

Introduction

The Japanese quail is fairly resistant to diseases, but clinical chemistry data can be useful aids for diagnosis and monitoring responses in birds, which often show no clinical signs (Fudge, 1997). To combat clinical and subclinical forms of poultry diseases, accurate and differential diagnosis of the diseases at early stages of infections is necessary. Measurement of haematological and serum biochemical parameters provides valuable information in this regards and is routinely used in human's and animal's medicines, but unfortunately due to lack of information, blood profile have not been widely used in avian medicine (Mushi *et al.*, 1999; Kral and Suchy, 2000). Avian blood differs in cells' characteristics from their mammalian counterpart (Smith *et al.*, 2000). Several factors including physiological (Alodan and Mashaly, 1999) and environmental conditions (Vecerek *et al.*, 2002, Graczyk *et al.*, 2003), nutrition (Odunsi *et al.*, 1999; Kurtoglu *et al.*, 2005), water and feed restriction (Galip, 1999; Al-Rawashdeh *et al.*, 2000; lhekumwure and Herbert, 2003), fasting (Lamosova *et al.*, 2004), age (Furlan *et al.*, 1999; Naziefy-Habibabadi, 1997; Seiser *et al.*, 2000), sex (Gabriel *et al.*, 2004; Cetin *et al.*, 2009) administration of drugs (Khan, *et al.*, 1994; Zaman *et al.*, 1995), anti-aflatoxin premixes (Oguz *et al.*, 2000) and continuous supplementations of vitamin E (Tras *et al.*, 2000) affect the blood profiles of poultry. Reference blood profiles of quails at different age in both sexes are essential for interpretation of haematological (Mushi *et al.*, 1999; Seiser *et al.*, 2000) and serum tests (Schlotz *et al.* 2009). However, insufficient information on reference blood profile values in quails has limited the application of this technique. Hence, this study was carried out to determine the blood profile

of male and female quails at various ages.

Materials and Methods

Site of Experiment

This experiment was carried out at the Directorate of University Farms (DUFARMS), Federal University of Agriculture Abeokuta. The area lies on latitude 7010'N and longitude 302'E, it is 76m above sea level and located in the tropical rainforest vegetation zone with an average temperature of 34.70C and relative humidity of 82% (Google Earth, 2014).

Source of Experimental Birds

A total of one hundred (100) unsexed day old Japanese quail chicks were purchased from a reputable hatchery in Abeokuta, Ogun State, Nigeria.

Source of Experimental Birds

Birds were brooded on deep litter for three weeks. Wood shavings were spread on the floor as litters to provide insulation and easy removal of waste. Stone pebbles were placed around the edge of the drinkers for the first week, in order to avoid the chicks from getting wet and drowned. Birds were fed diets shown in Table 1. Fresh and clean water were supplied regularly. Sexing was done at the third week by observing the differences in the breast feather colour.

Blood Samples Collection

Three (3) birds from each sex were randomly selected for haematological and serum biochemical analysis at the 3rd, 5th, 7th and 9th week of age. 3ml (1.5ml each for haematology and serum biochemistry) of blood were collected by severing the jugular vein of the selected birds in the morning (8.00-

Table 1: Gross composition of the experimental diet

Ingredients	0-6weeks	6weeks and above
Maize	51.05	62.65
Soya bean cake	27.86	19.16
Groundnut cake	9.29	6.39
Fish meal	3.00	3.00
Wheat offal	5.00	5.00
Bone meal	2.50	2.50
Limestone	0.50	0.50
Lysine	0.10	0.10
Methionine	0.15	0.15
Common salt	0.30	0.30
Vitamin premix	0.25	0.25
Total	100.00	100.00
Calculated analysis		
Crude protein (%)	24.10	20.2
M. E. (Kcal/kg)	12.19	12.72
Ether Extract (%)	4.0	4.0
Crude fibre (%)	3.80	3.3
Calcium (%)	1.2	1.20
Phosphorus (%)	0.60	0.5

Vitamin/premix contains Vit. A - 4,000,000.00 IU; Vit D3 - 800,000.00IU; Vit. E - 9,200.00mg; Vit K - 800.00mg; thiamin (B1) - 720.00mg; Riboflavin (B2) - 2,000.00mg; Pyrodixine (B2) - 1,200.00mg; Vit. B12 - 6.00mg; Biotin - 24.00mg; Niacin - 11,000.00gm; Panthothenicacid - 3,000.00gm; Folicacid - 300.00gm; Chlorinechloride - 120,000.00gm; Iron - 8,000.00gm; Manganese - 16,000.00gm; Copper - 1,200.00gm; Zinc - 12,000.00gm; Cobalt - 80.00gm; Iodine - 400.00gm; Selenium - 80.00gm; Antioxidants - 500.00mg

9.00 a.m). Blood samples for haematology were collected in a clean Ethylene Diamine Tetra Acetic (EDTA) bottles to prevent blood samples from coagulating while blood samples for serum analysis were collected in sterile bottles.

Determination of Haematological Parameters

Haematological parameters; haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) and others such as Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were estimated by

following procedures described by Jain (1986). Mean Corpuscular Volume was estimated by calculation using a standard formula (Jamie and Howlett, 2008).

Serum total protein, albumin, globulin, glucose, cholesterol were determined spectrophotometrically (Chemito Spectrosan 2600) according to the method of Tietz (1995) as described in Randox(R) diagnostic kit manual. Alanine and Aspartate Aminotransferase were determined spectro-photometrically according to the method of Reitman and Frankel (1957), using commercial Randox kit.

Statistical Analysis

The experiment was arranged in a 4x2 factorial layout. Data collected were subjected to completely Randomised design using SPSS version 16 for Windows. Significant ($p < 0.05$) differences among treatment means were separated using Duncan Multiple Range Test of the software

Results

Table 2 presents the main effects of age and sex on haematological indices of Japanese quail. Age of birds did not affect ($P > 0.05$) most of the haematological parameters except the haemoglobin and Mean corpuscular haemoglobin concentration (MCHC). Packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) increased ($P > 0.05$) numerically with increased age. Haemoglobin was lowest ($P < 0.05$) at the third week (12.50g/dl), highest ($P < 0.05$) at the 9th week (16.40g/dl) while values at 5th (13.95g/dl) and 7th (15.35g/dl) week were similar ($P > 0.05$) and comparable to other weeks. A linear increase ($P < 0.05$) in MCHC was recorded with increased age.

All haematological parameters were not significantly ($P > 0.05$) different between the sexes.

The effects of interaction between age and sex on haematological indices of Japanese quail are presented in Table 3. All determined haematological indices did not indicate any significant ($P > 0.05$) effect of interaction between age and sex in quails except ($P < 0.05$) for the PCV of male quails which was highest

Table 2: Main effects of age and sex on haematological indices of Japanese quails

Parameters	Age (weeks)				SEM	Sex		
	3	5	7	9		Female	Male	SEM
PCV(%)	39.25	43.50	47.00	49.25	2.96	44.75	44.75	2.09
Haemoglobin(g/dl)	12.50 ^b	13.95 ^{ab}	15.35 ^{ab}	16.40 ^a	1.01	14.51	14.59	0.72
RBC (x 1012 μ /L)	3.15	3.40	3.75	3.80	0.33	3.50	3.55	0.23
WBC(x 103 μ /L)	15.60	17.65	20.30	21.13	1.43	18.26	19.08	1.01
Heterophil (%)	34.25	33.75	34.00	35.00	1.91	35.25	33.25	1.35
Lymphocytes (%)	63.75	65.00	64.25	64.25	1.63	63.50	65.13	1.15
Eosinophil (%)	0.50	0.50	0.50	0.25	0.38	0.25	0.63	0.27
Basophil (%)	1.0	0.25	0.50	0.25	0.31	0.38	0.63	0.22
Monocyte (%)	0.5	0.50	0.75	0.25	0.25	0.63	0.38	0.18
MCV (fl)	124.50	131.00	125.5	130.00	6.65	128.63	126.88	4.71
MCH (pg)	40.00	42.00	41.00	43.50	2.32	41.75	41.50	1.64
MCHC (g/dl)	32.00 ^b	32.25 ^b	32.75 ^{ab}	33.50 ^a	0.35	32.50	32.75	0.25

Table 3: Effect of interaction between age and sex on haematological indices in quails

Parameters	Female				Male				SEM
	3	5	7	9	3	5	7	9	
PCV(%)	42.00 ^{ab}	42.00 ^{ab}	47.50 ^{ab}	47.50 ^{ab}	36.50 ^b	45.00 ^{ab}	46.50 ^{ab}	51.00 ^a	4.18
Haemoglobin(g/dl)	13.40	13.45	15.40	15.80	11.60	14.45	15.30	17.00	1.43
RBC (x 1012 μ /L)	3.25	3.20	3.85	3.70	3.75	3.60	3.65	3.90	0.46
WBC(x 103 μ /L)	15.65	17.60	20.20	19.60	15.55	17.70	20.40	22.65	2.02
Heterophil (%)	38.00	35.00	33.50	34.50	30.50	32.50	34.50	35.50	2.70
Lymphocytes (%)	60.50	63.50	65.00	65.00	67.00	66.50	63.50	63.50	2.29
Eosinophil (%)	0.50	0.50	0.00	0.00	0.50	0.50	1.00	0.50	0.53
Basophil (%)	0.50	0.00	0.50	0.50	1.50	0.50	0.50	0.00	0.43
Monocyte (%)	0.50	1.00	1.00	0.00	0.50	0.00	0.50	0.50	0.35
MCV (fl)	129.00	133.00	123.50	129.00	120.00	129.00	127.50	131.00	9.40
MCH (pg)	41.50	42.50	40.00	43.00	38.50	41.50	42.00	44.00	3.28
MCHC (g/dl)	32.00	32.00	32.50	33.50	32.00	32.50	33.00	33.50	0.50

Means with different superscripts on the same row differ significantly ($P < 0.05$)

at the 9th week (51.00%) and least at the 3rd week (36.50%).

The main effects of age and sex on serum biochemistry of quail are presented in Table 4. Blood total protein and albumin values decreased ($P < 0.05$) with increasing age. Globulin was highest ($P < 0.05$) at the 3rd week (2.10g/dl). Blood glucose was similar ($P > 0.05$) across the ages and ranged from 130.75 to 141.25mg/dl. Cholesterol was highest ($P < 0.05$) at the 7th week (135.25mg/dl), however, similar ($P > 0.05$) values were obtained at the 3rd and

9th week though the least among the ages. Blood calcium increased ($P < 0.05$) with increase in ages. Aspartate transaminase and Alanine transaminase were significantly ($P < 0.05$) highest at the 9th week (59.25 and 35.50 μ /L, respectively).

Only blood cholesterol was significantly ($P < 0.05$) different between female (131.88mg/dl) and male (125.75mg/dl) quails, other serum parameters were similar in both sexes.

Table 4: Main effects of age and sex on serum biochemistry of Japanese quails

Parameters	Age (weeks)				SEM	Sex		
	3	5	7	9		Female	Male	SEM
Total protein(g/dl)	5.10 ^a	4.40 ^{ab}	4.38 ^{ab}	4.10 ^b	0.22	0.16	0.29	0.16
Albumin(g/dl)	3.00 ^a	2.92 ^a	2.58 ^{ab}	2.28 ^b	0.16	2.61	2.78	0.11
Globulin(g/dl)	2.10 ^a	1.48 ^b	1.80 ^{ab}	1.83 ^{ab}	0.16	1.78	1.83	0.12
Glucose(mg/dl)	130.75	141.25	132.00	136.00	5.15	133.5	136.5	3.64
Cholesterol(mg/d)	125.25 ^b	130.00 ^{ab}	135.25 ^a	124.25 ^b	2.62	131.88 ^a	125.75 ^b	1.85
Calcium(mg/dl)	7.28 ^b	7.45 ^b	8.30 ^{ab}	9.55 ^a	0.60	8.50	7.79	0.43
Urea(mg/dl)	1.93	1.95	1.80	2.00	0.16	0.09	0.10	0.11
AST (μ /L)	41.75 ^b	46.75 ^b	48.75 ^b	59.25 ^a	2.53	49.75	48.50	1.79
ALT (μ /L)	23.25 ^b	28.00 ^b	36.75 ^a	35.50 ^a	2.13	28.86	32.88	1.51

Means with different superscripts on the same row differ significantly ($P<0.05$)

Table 5: Effect of interaction between age and sex on serum biochemistry in quails

Parameters	Female				Male				SEM
	3	5	7	9	3	5	7	9	
Total protein (g/dl)	4.60 ^{abc}	3.95 ^{bc}	4.50 ^{bc}	4.50 ^{bc}	5.60 ^a	4.85 ^{ab}	4.25 ^{bc}	3.70 ^c	0.31
Albumin (g/dl)	2.80 ^a	2.55 ^{ab}	2.50 ^{ab}	2.60 ^{ab}	3.20 ^a	3.30 ^a	2.65 ^{ab}	1.95 ^b	0.23
Globulin (g/dl)	1.80 ^{ab}	1.40 ^b	2.00 ^{ab}	1.90 ^{ab}	2.40 ^a	1.55 ^b	1.60 ^{ab}	1.75 ^{ab}	0.23
Glucose (mg/dl)	128.00	141.00	133.50	131.50	133.50	141.50	130.50	140.50	0.28
Cholesterol (mg/d)	130.50 ^{abc}	135.00 ^{ab}	131.00 ^{abc}	131.00 ^{abc}	120.00 ^{cd}	125.00 ^{bcd}	140.50 ^a	117.50 ^d	3.70
Calcium (mg/dl)	7.85	7.85	8.85	9.45	6.70	7.05	7.75	9.65	0.85
Urea (mg/dl)	1.90	1.85	1.55	1.90	1.95	2.05	2.05	2.10	0.23
AST (μ /L)	39.00 ^b	44.00 ^b	47.00 ^b	69.00 ^a	44.50 ^b	49.50 ^b	50.50 ^b	49.50 ^b	3.57
ALT (μ /L)	19.50 ^c	27.00 ^{bc}	34.00 ^{ab}	35.00 ^{ab}	27.00 ^{bc}	29.00 ^{abc}	39.50 ^a	36.00 ^{ab}	3.01
MCV (fl)	129.00	133.00	123.50	129.00	120.00	129.00	127.50	131.00	9.40
MCH (pg)	41.50	42.50	40.00	43.00	38.50	41.50	42.00	44.00	3.28
MCHC (g/dl)	32.00	32.00	32.50	33.50	32.00	32.50	33.00	33.50	0.50

Means with different superscripts on the same row differ significantly ($P<0.05$)

The effects of interaction between age and sex on blood serum parameters in quail are presented in Table 5. Values were significantly ($P<0.05$) different across the treatments except ($P>0.05$) blood glucose, calcium and uric acid. The highest ($P<0.05$) and the least total protein of male quails were recorded at the 3rd (5.60g/dl) and 9th week

(3.70g/dl) respectively. Albumin was similar in female and male at 3rd week and male at 5th week though higher ($P<0.05$) when compared to other groups. Blood glucose ranged from 128.00 to 135.00mg/dl.

Blood calcium and uric acid ranged from 6.7- 9.45mg/dl and 1.55-2.05mg/dl, respectively. Female quails had the highest ($P<0.05$) AST

(69.00 μ /L) at the 9th week while they were similar ($P>0.05$) among other groups. ALT was comparable at the 7th and 9th week in both sexes.

Discussion

The range of packed cell volume (39.25-49.25%) across the ages in this study was higher than the range (30.01- 39.80%) reported by Aina and Ajibade (2014) in 1-6weeks old quails. This variation could be the age difference as this study compared PCV among 3- 9weeks old quails. However, PCV values reported by Akade *et al.* (2012) in 6weeks old quails was similar to those obtained in this study between the 5th and 7th week. Ali *et al.* (2012) reported that there was no difference in PCV among 5, 6 and 7weeks old quails which is consistent with the result of this study. Haemoglobin increased with increasing age in this study but PCV, red blood cell (RBC) and white blood cell (WBC) only increased numerically with increasing age. This is in consonance with the findings of Aina and Ajibade (2014) also reported no effect of age on the RBC, WBC and PCV. However, Rajil *et al.* (2000) reported increase in RBC with advancement in age. Similar haemoglobin values reported by Aina and Ajibade (2014) in 1-6 weeks old quail as against marked difference in haemoglobin obtained in this study could be as a result of age interval (weekly vs fortnight). Lymphocytes are the leukocyte component with the highest percentage which is consistent with the report by Bounous *et al.* (2000).

Birds' haematology did not differ between sexes in this study. Contrarily, Addas *et al.* (2012) observed a significant sex effect for PCV, RBC, WBC, MCHC and MCV with males having the highest PCV, RBC and MCV in chickens. Higher values in males than females in parameters relating to RBCs were also reported in pheasants (Hauptmanova *et al.*, 2006), geese (Lazar *et al.*, 1991), Japanese quails (Mihailov *et al.*, 1999), budgerigars (Itoh, 1992), chickens (Oladele *et al.*, 2000) and guinea fowls (Oladele *et al.*, 2005; Obinna *et al.*, 2011).

The highest total protein and albumin obtained at the 3rd week of age in this study could be as a result of high demand for amino acids for intensive somatic growth. Szabo *et al.*

(2005) and Filipowic *et al.* (2007) stated that increased blood plasma protein and albumin during the growing stage in chicken is probably due to direct consequence of high demand for amino acids which are utilized for very intensive somatic growth. However, this result is at variance with increased concentrations of total protein and cholesterol as the age increases in quails reported by Nazifi *et al.* (2000). Blood plasma protein did not reveal sex influence and this is corroborated by work of Nazifi *et al.* (2000) in male and female quails.

Blood glucose was similar in both sexes in this study and this is consistent with similar blood serum glucose in close bred male and female Japanese quail studied by Jatoi *et al.* (2013). However, Rajil *et al.* (2000) indicated that female quails had a comparatively higher blood glucose at various ages which is at variance with the result of this study. Increased blood cholesterol obtained between 3-7weeks in this study was similar to earlier reports (Nazifi *et al.* 2000; Ali *et al.*, 2012) in quails at different ages.

Higher blood cholesterol in female quails than the male in study is corroborated by the report by Scholtz *et al.* (2009) in quails and Elagib *et al.* (2012) in three indigenous chicken types in Sudan. Soliman and Huston (1974) stated that types of fat used in the diet, genetic differences, sex differences and age differences affect plasma cholesterol. Differences in blood cholesterol could be attributed to changes in physiology of metabolism in female birds due to increased synthesis of triglycerides, phospholipids and cholesterol during the laying period (Walzem *et al.*, 1999).

Blood calcium increased with age and this agrees with report by Ali *et al.* (2012) in quails and Piotrowska *et al.* (2011) in broiler chicken. Blood calcium values fall within the range (8.60- 21.64mg/dl) reported by Ali *et al.* (2012).

Conclusion

Based on the result of this study, it can be concluded that haemoglobin increased with advancement in age while other haematological parameters were not affected. Sex did not

influence haematological parameters in quails; however, female quails had higher cholesterol than male quails.

Impact

This study provides valuable information regarding the haematological and serum biochemical properties of male and female quails at different ages. This information are relevant in diagnosis of diseases and monitoring of health of quails which are novel poultry in Nigeria, hardy and rarely show clinical signs.

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THE EVALUATION OF INTRANASAL *Lactobacillus fermentum* AND *Mannheimia haemolytica* BACTERINE IN THE CONTROL OF *Mannheimia haemolytica* INFECTION IN GRASSCUTTER (THRYONOMYS SWINDERIANUS TEMMINCK, 1827)

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Abstract

This study evaluates the effect of recombinant *Mannheimia haemolytica* bacterine and *Lactobacillus fermentum* intranasal administration on experimental pneumonic Mannheimiosis in grasscutters (*Thryonomys swinderianus* Temminck, 1827). This investigation involved apparently healthy adult grasscutters that were divided into groups A, B, C, D, E, F, G and H of three (3) grasscutters each with group H serving as the control. Group A (vaccinated twice with bacterin and infected), group B (vaccinated once with bacterin and infected), group C, E (vaccinated, inoculated with *Lactobacillus fermentum* and infected), group D (inoculated with *Lactobacillus fermentum* alone), group F (infected), group G (grasscutters infected and later inoculated with *Lactobacillus fermentum*) and lastly group H (control). The mortality pattern, pathology of the respiratory tract and nasal bacterial load were assessed using standard methods. The result showed that intranasal application of *Lactobacillus fermentum* and or recombinant *Mannheimia haemolytica* bacterine reduces the nasal bacterial load, pathology and mortality pattern associated with experimental *Mannheimia haemolytica* infection in the grasscutter hence they could be useful in the control of respiratory disorders in grasscutters.

Keywords: grasscutter, bacterin, experimental, respiratory, infection

L'ÉVALUATION DE L'APPLICATION INTRANASALE DE LA BACTÉRINE À BASE DE *Lactobacillus fermentum* ET DE *Mannheimia haemolytica* DANS LE CONTRÔLE DE L'INFECTION À *Mannheimia haemolytica* CHEZ L'AULACODE (THRYONOMYS SWINDERIANUS TEMMINCK, 1827)

Résumé

La présente étude évalue l'effet de l'administration intranasale de la bactérine à base de *Mannheimia haemolytica* et de *Lactobacillus fermentum* recombinantes sur la Mannheimiose pulmonaire expérimentale chez les aulacodes (*Thryonomys swinderianus* Temminck, 1827). L'étude a utilisé des aulacodes adultes apparemment en bonne santé, qu'elle a répartis en groupes A, B, C, D, E, F, G et H de trois (3) aulacodes chacun, le groupe H servant de témoin. Le Groupe A (vacciné deux fois avec la bactérine et infecté), le Groupe B (vacciné une fois avec la bactérine et infecté), le Groupe C, E (vaccinés, inoculés avec *Lactobacillus fermentum* et infectés), le Groupe D (inoculé avec *Lactobacillus fermentum* uniquement), le Groupe F (infecté), le Groupe G (aulacodes infectés et plus tard inoculés avec la *Lactobacillus fermentum*) et enfin le Groupe H (témoin). Le schéma de mortalité, la pathologie des voies respiratoires et la charge bactérienne nasale ont été évalués en utilisant des méthodes standard. Le résultat a montré que l'application intranasale de la *Lactobacillus fermentum* et ou de la bactérine à *Mannheimia haemolytica* recombinante réduit la charge bactérienne nasale, la pathologie et le schéma de mortalité associée à l'infection expérimentale avec *Mannheimia haemolytica* chez les aulacodes. Par conséquent, ces espèces de bactéries pourraient être utiles dans la lutte contre les troubles respiratoires des aulacodes.

Mots-clés : aulacode ; bactérine ; expérimentale ; respiratoire ; infection

Introduction

The cane rat or grasscutter (*Thryonomys swinderianus*) is, a wild hystricomorphic rodent, widely distributed in the African sub – region and is often exploited in most areas as source of animal protein (Opara and Fagbemi, 2010). Being the most preferred and most expensive meat (consumed as bushmeat) in West Africa (Asibey and Addo, 2000), it contributes to both local and export earnings (Ntiamoa-Baidu, 1998) and is often an alternative source of the animal protein that complement the conventional livestock like sheep, cattle and goat (Byanet *et al.* 2009) especially in rural areas.

The high demand for grasscutter meat and the economic benefit that accrue from its sale has resulted in aggressive hunting and high curiosity by researchers to investigate their disease pattern especially with renewed vigor to domesticate grasscutter (National Research Council, 1991; Addo, 2002). This renege vigour will make the meat with an excellent taste and comparatively higher nutritional value more readily available. It will also reduce the environmental destruction that accompanies its collection from the wild (Asibey & Eyeson, 1973).

The previous unsuccessful attempts to domesticate this rodent (Opara *et al.*, 2006) may be attributable to poor understanding of its biology, lack of veterinary care and other management factors. Though earlier studies have emphasized on the ethology (Codjia, 1985; Holzer *et al.*, 1985), feeding, pathology (Akomedi, 1988), selection systems (Senou *et al.*, 1992), reproduction (Adjanohoun, 1988, 1992a, b) and the technical feasibility of grasscutter farming are aspects of lesser though they are essential areas for a successful domestication (Adoun, 1992a, Yeboah and Adamu 1995).

Although Jori and Chardonnet (2001) reported significant improvement of some production parameters such as prolificacy of females, growth rate, number of weaned kits per litter, due to genetic selection and improved diet. Nevertheless, very few information exist in literatures on disease conditions in grasscutter (Tondji *et al.*, 1992; Schrage and Yewadan, 1995;

Adu, *et al.*, 2000)

With increasingly interest in the domestication of grasscutter in many African countries, the need for understanding of disease process of farmed grasscutter cannot be over emphasised (Jori, *et al.*, 1995). Many authors had identified gastroenteritis and pneumonia as one of the primary cause of death in grasscutters (Akomedi, 1988; Tondji *et al.*, 1992, Adu, *et al.*, 2000 Jori, *et al.*, 2001 and Adu, 2002) hence, the need to explore means of alleviating respiratory infection in grasscutter (Oboegbulem and Okoronkwo 1990, Muller 1995). Standard approach to control of pneumonia in domestic animals had been with the use of antibiotics and vaccines. With the emergence of antibiotic resistant strains, most control approaches now focus on the use of intranasal vaccines while that of application of probiotics in the respiratory tract is relatively a new approach. This study therefore sought to explore the influence of *Lactobacillus fermentum* as a possible nasal probiotics and recombinant Mannheimia hemolytica bacterine on experimental pneumonic Mannheimiosis in grasscutters (*Thryonomys swinderianus* Temminck, 1827).

Materials and Methods

Study Area

The study was carried out at the domestication unit of the Department of Wildlife and Ecotourism Management within the University of Ibadan, Ibadan, Nigeria. The University of Ibadan is situated some kilometers to the north of the city of Ibadan at Latitude 7°26" North and Longitude 3° 54" East at a mean altitude of 277 meters above sea level. The annual rainfall is approximately 1,220mm (48") most of which falls between the months of April and October inclusive, given a predominantly dry season from November to March.

Animals

Twenty four (24), Twelve month old grasscutters procured from Tropical farm domestication unit, Imo State were used for the study. The farm is a well established farm

that major in the breeding and domestication of the animal. All animals for the study belonged to a selected strain of animals adapted to captivity. The grasscutters were housed in the Department of Wildlife and Ecotourism management domestication unit. They were kept in cages constructed for them at the unit. The grasscutters were initially quarantined and maintained to acclimatize to the new housing environment. The grasscutter were provided with feed and water ad libitum. All existing housing and management techniques utilized for efficient breeding of stock in the domestication unit were utilized (reference). The grasscutters used for this research work were fed mainly with elephant grass (*Pennisetum purpureum*) and with compounded feed.

Challenge Bacteria

0.5 ml of pure culture (107 CFU) of a 4 hour log phase culture of *Mannheimia haemolytica* A2 in brain infusion broth was used intranasally to challenge the grasscutters.

Isolation and preparation of *Lactobacillus fermentum*

The *Lactobacillus fermentum* used was obtained from the Elephant grass stem (cane part) of *Pennisetum purpureum* consumed by the grasscutters. de Man Rogosa and Sharpe (MRS) medium was used to isolate *Lactobacilli* strains (De Mann *et al.*, 1960; Christian, 1970). Sliced stems of Elephant grass (*Pennisetum purpureum*) were collected from the feeding stock of the grasscutters, placed in conical flask containing distilled water to form a concentration pulp. Using different sterile 2.0ml pipette, 1 ml of 10¹ to 10¹⁰ of the serial dilution of the various samples were plated out by mixing with MRS agar medium in McChartney bottles and poured aseptically into sterile Petri dishes. The plate was swirled round for even distribution of inoculums and settling of the agar. After solidifying, the Petri-dishes were incubated in anaerobic jars using BTL gas Park hydrogen and carbon dioxide generators (BBL Bacton, Dickson and Co; USA, MD 21030). After the incubation period, the plates were observed for bacterial growth and the colonies were randomly selected. Isolates

were sub-cultured repeatedly to obtain pure cultures of the isolates. Characterization of isolates was by macroscopic, microscopic, and physiological and biochemical tests. Identification to be *Lactobacillus fermentum* was done using API 50 CHL Medium. The isolated *Lactobacilli* were maintained in MRS broth with 12% (w/v) glycerol and inoculated at 30°C until growth becomes visible. The stock cultures were stored at 4°C for subsequent use.

Experimental Infection of Grasscutters

The grasscutters were divided into groups A – H. Groups A to G were the experimental groups and group H was the control. Each group consists of three grasscutters each.

Mortality pattern, nasal bacterial load and pathological changes after challenge

The mortality, nasal bacterial load and pathological changes in the respiratory tracts were assessed after challenge

Results

There was no mortality in all the groups however; nasal discharges were noticed in *Mannheimia hemolytica* infected group F. The gross pathological changes were insignificant in the respiratory tract of all the groups except Group F where the nasal septae were hyperemic.

Result from this group showed that the MH load dropped from the initial load when the bacterin was given. At the 7th week the load was reduced. In group B, where the bacterine was administered once by 3rd week the nasal load increased at the sixth (6th) and seventh (7th) week.

In group (C), the inoculation of *Lactobacillus fermentum* (LAB) in the second (2nd week) reduced nasal bacterial at the fourth (4th) week while after infection, the load increased at the sixth (6th) and seventh (7th) week.

In group (D), the grasscutters were inoculated with *Lactobacillus fermentum* (LAB) alone. The initial microbial load was high by the first (1st week). By the fourth (4th) sixth (6th)

Table 1: The experimental Design

	Description	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8
A	(2 X Bacterin)	Bacterin		Bacterin			InfectedMH		Slaughtered
B	Bacterin+MH			Bacterine			InfectedMH		Slaughtered
C	(LAB+MH)			LAB			InfectedMH		Slaughtered
D	(LAB Alone)			LAB					Slaughtered
E	(LAB+MH)					LAB	InfectedMH		Slaughtered
F	InfectedAlone)						InfectedMH		Slaughtered
G	(Infected+LAB)						InfectedMH	LAB	Slaughtered
H	Control								Slaughtered

LAB- *Lactobacillus fermentum*.

Table 2: Nasal bacterial load (X 107 CFU) of grasscutters in the different treatment groups

	Description	Initial	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8
	Bacterial load									
A	(2 X Bacterin)	18	7	6.5	-	-	-	4.5	3.5	Slaughtered
B	Bacterin+MH	8	-	5	7	-	-	30	40	Slaughtered
C	(LAB+MH)	9.5	-	-	6	5	-	12.5	21	Slaughtered
D	(LAB Alone)	14.5	-	-	-	13	-	8.5	4.5	Slaughtered
E	(LAB+MH)	9.5	-	-	-	-	-	9.5	5	Slaughtered
F	InfectedAlone)	13.5	-	-	-	-	-	27.5	25.5	Slaughtered
G	(Infected+LAB)	6.5	-	-	-	8.5	-	6	4	Slaughtered
H	Control	14.5	-	-	-	12.5	-	13	14.5	Slaughtered

and seventh (7th) week after inoculation, the microbial load had reduced. In group (E), the grasscutters were inoculated with *Lactobacillus fermentum* (LAB) at the fourth (4th) week, a week before infection. The initial microbial load was high by the first (1st week) but reduced by the sixth (6th) and seventh (7th) week.

In group (F), the grasscutters were infected with *Mannheimia haemolytica* at the fifth (5th) week. The initial microbial load was high by the first (1st week) progressed to a higher level by the Sixth week and by the seventh (7th). In group (G), the grasscutters were infected with *Mannheimia haemolytica* at the fifth (5th) week and inoculated at the sixth (6th) week. The initial microbial load was low by the first (1st week) progressed to a higher level by the fourth week. In group (H), the grasscutters were not infected but was used as control. Findings from this group showed that *Mannheimia haemolytica* was part of the normal nasal bacterial flora of the animals.

Discussion

The study showed that *Mannheimia haemolytica* (MH) bacterine and *Lactobacillus fermentum* have potential to prevent pneumonia through the reduction in MH load in respiratory tract of grasscutters. MH bacterine used in this study had been successfully used to curb pneumonia in Boer goats in Sabah, Malaysia (Sabri *et al.*, 2013) but has been found not to be protective against naturally occurring pneumonia in Nigerian goats (Emikpe *et al.*, 2013b). In this study however, it was observed to be protective against experimental Mannheimiosis in grasscutter with reduction in nasal bacterial load and lesions.

This investigation also gave an insight to the possible use of *Lactobacillus fermentum* for the control of respiratory infection in grasscutter. The use of *Lactobacillus fermentum* in this study showed it's probable use as a nasal

probiotics with its ability to effectively reduce nasal MH load and respiratory lesion when applied few days after infection than when its after some weeks, this might be related with competition for attachment and eventual reduction of MH colonization since attachment of MH to the basement membrane of the respiratory tract epithelium is an important step in the Mannheimiosis (Jasni *et al.*, 1991, Emikpe *et al.*, 2013a, Emikpe *et al.*, 2014).

The use of probiotics is an established protocol to control of enteropathy in domestic animals including laboratory animals, however there had been no report of such in the respiratory tract for the control of respiratory infection in domestic animals. The reduction in the bacterial load after the application of the bacterine and *Lactobacillus fermentum* when used separately further support the fact that MH bacterine and administration of *Lactobacillus fermentum* is capable of reducing the colonization of the challenge bacteria and subsequently avert the pathology associated with MH and this in turn resulted in 100% survival recorded in all the groups except the positive control group F

In conclusion, this investigation gives preliminary information on the use of MH bacterine and *Lactobacillus fermentum* in the control of respiratory tract infection of grasscutters. It also showed that intranasal *Lactobacillus fermentum* is useful when the respiratory infection just commences in grasscutter.

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RESPONSE OF BROILER CHICKENS TO FEED SUPPLEMENTED WITH CLAY-BASED NANO GROWTH PROMOTER

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Abstract

One of the most critical issues in human food safety and health is the public outcry against the potential health risks and environmental problems associated with excessive use of feed antimicrobials, growth hormones and synthetic pharmaceuticals. An eight week study was carried out to evaluate the effects of MFeed®, a nanotechnology growth promoter as feed additive in the diets of broiler chickens. Two hundred, day old Marshal broiler chicks were allotted in a completely randomised design to five dietary treatments supplemented with MFeed® at 0, 1, 2, 3, 4 and 5 g/kg of the basal diet respectively. Data were collected on growth performance, intestinal pH, haematological and serum parameters of broiler chickens fed the experimental diets at the fourth and eighth week. Body weight, survivability, and the health of broiler chickens improved significantly ($P < 0.05$) with increased MFeed® in the diets. From this study it can be concluded that supplementation of MFeed® in broiler chickens diets will improve growth and the health status.

Keywords: broilers, nano growth promoter, performance, blood parameters

REPONSE DES POULETS DE CHAIR AUX ALIMENTS ENRICHIS AVEC UN PROMOTEUR DE CROISSANCE A BASE DE NANO-ARGILES

Résumé

L'un des problèmes les plus critiques en matière de sécurité sanitaire des aliments et de santé humaine est le cri d'alarme public contre les risques sanitaires potentiels et les problèmes environnementaux liés à l'utilisation excessive d'antimicrobiens, d'hormones de croissance et de produits pharmaceutiques synthétiques dans les aliments. Une étude de huit semaines a été réalisée dans le but d'évaluer les effets de MFeed®, un promoteur de croissance basé sur la nanotechnologie, comme additif alimentaire dans l'alimentation des poulets de chair. Deux cents poussins de chair Marshal d'un jour ont été répartis dans un dispositif complètement aléatoire à cinq traitements alimentaires supplémentés avec MFeed® à 0, 1, 2, 3, 4 et 5 g / kg du régime alimentaire de base. Les données ont été recueillies sur les performances de croissance, le pH intestinal, les paramètres hématologiques et sériques des poulets de chair soumis aux régimes expérimentaux aux quatrième et huitième semaines. Le poids corporel, la possibilité de survie et la santé des poulets de chair se sont améliorés de manière significative ($P < 0,05$) à la suite d'une augmentation de l'apport de MFeed® dans les régimes. Sur la base de cette étude, on peut conclure que la supplémentation des régimes des poulets de chair avec MFeed® permettra d'améliorer la croissance et l'état de santé.

Mots-clés : poulets de chair, promoteur de croissance nano, performance, paramètres sanguins

Introduction

Feed stuffs used in animal production are often contaminated with pathogenic bacteria which in turn, adversely affect growth and productivity of animals. Litter materials use under deep litter system are not naturally sterile, the pathogenic bacteria found in them was an indication of their potential to contain injurious organisms (Asaniyan *et al.*, 2007).

To maximize the genetic potential of broilers chickens, they must be free from diseases as well as have diets that meet their nutritional requirements for optimal growth and production.

Antimicrobial growth promoters are antimicrobial supplements added to animal feed to enhance growth and improve feed efficiency of food animals. Antibiotics have been given at sub therapeutic dosage as feed additives to stabilize the intestinal microflora and improve the general performances of animals (Dibner and Richards, 2005). As a result of the growing concern of the impact of antimicrobials used in animals on human health and food safety, the World Health Organization (WHO) published the WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food (WHO, 2000).

According to Aarestrup (2003), pathogenic bacteria resistant to a number of antimicrobial agents emerged worldwide in the 1980s, as these were detected reports recommending a ban on antimicrobial use in food animals as a precautionary measure to consumer concerns over food safety and voluntary stoppage of the use of all antimicrobial growth promoters is required. It was reported that antibiotic resistance genes can be and are transmitted from animal to human microbiota (Greko, 2001). Monitoring and identifying resistance mechanisms and their dissemination into the food chain were recently reviewed by Roe and Pillai (2003).

There is an increasing interest in finding alternatives to antibiotics in poultry production. These alternatives include acidifiers (organic acids), prebiotics, probiotics, enzymes, herbal products, microflora enhancers, and immuno-modulators (Waldroup *et al.*, 2003).

The focus of alternative strategies has been to prevent resistance of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance are improved (Ravindran, 2006). New growth promoter exploitations, especially on biodegradable polymer nanoparticles, have attracted more attention recently.

Nanotechnology is an innovative technology, which is used to create materials and change structure, enhanced quality and texture of foodstuffs at the molecular level by synthesis at the atomic level (Carmen *et al.*, 2003). With the development of nanotechnology which manipulates matter on nano-scale with certain clays known to have developed high chemical reactivity in the fields of catalysis. This is a process whereby the structures of these clays were transformed in order to increase the accessibility to the catalytic active sites of the internal structure and develop more contact surfaces. Through the nanotechnology modification of natural montmorillonite, a new additive-montmorillonite nanocomposite (MFeed[®]) has been developed. The aim of this study was to evaluate the effect of Mfeed[®] as growth promoter on performance and blood parameters of broiler chickens fed rations supplemented Mfeed[®].

Materials and Methods

A total of two hundred (200) unsexed day old Marshal Strain broiler chicks were randomly allotted into five treatments in a completely randomized design of thirty six birds per treatment with four replicates.

Test feed additive

The montmorillonite nanocomposite (MFeed[®]) used in this study was obtained from Olmix, France. MFeed[®] supplement is a biocatalyst, consists of sea lectuce, montmorillonite, diatomaceous earth, yeast extracts, seaweed extracts and essential oils.

Experimental diets

Commercial broiler starter and finisher mashes were purchased from a reputable feed miller, composition of the diets were shown in

Table 1. The dietary treatments consisted of the basal diet as control (0), 1, 2, 3 and 4 g/kg MFeed® added to the basal diet.

Data collection

Growth parameters

Body weights of broilers were measured on weekly basis, feed intake was measured at the same time, feed conversion was calculated accordingly and mortality was recorded..

Haematological and serum parameters

At the end of the fourth and eighth week, two birds per replicate were punctured from the wing vein to obtain 5mls of blood from which 2ml of each was collected into bottles with ethylene diamine tetra acetic acid (EDTA) for haematological parameters. Microhaematocrit method was used to determine packed cell volume (PCV) and cyanmethaemoglobin method was used to determine haemoglobin. White blood cell (WBC) and red blood cell (RBC) were determined using Neubauer haemocytometer. While those in bottles without EDTA were processed for serum analysis as described by Lamb (1981). The serum was used for the determination of total serum protein, albumin, globulin, uric acid, creatinine, aspartate aminotransferase (AST) and alanine transaminase (ALT).

Intestinal pH

The birds used for haematological and serum parameters at the fourth and eighth week were sacrificed by cervical dislocation. The duodenum and jejunum contents were collected in 90 ml sterilized saline (1: 10 dilution) and pH was determined using a pH probe which was inserted into the solution and a reading was recorded once the pH is stable.

Statistical Analysis

The data obtained were analyzed using the General Linear Model procedure of Statistical Analysis System 9.1 version (2000) and Polynomial contrast analysis. Significant means were compared using Duncan's multiple range test of same package.

Results

At the starter and finisher phases as indicated in Table 2 final weight, weight gain, feed intake, feed conversion ratio and mortality were significantly ($P<0.05$) affected by the dietary treatments. Supplementing MFeed® at 2g/kg and above ($P<0.05$) increased body weight at the starter and finisher phases while; average feed intake, intestinal pH and mortality decreased with increased supplementation level. Feed conversion ratio improved with increased MFeed® in the diets. The effects of dietary treatment on haematological parameters of broiler chickens as presented in Table 3 showed significant ($P<0.05$) increase in the values obtained in packed cell volume, haemoglobin, red blood cells. Packed cell volume, haemoglobin and red blood cell were elevated in broilers fed MFeed® at 2g/kg and above at the 4th and 8th week. White blood cell was markedly ($P<0.05$) reduced in broilers fed higher doses of MFeed®. Packed cell volume, haemoglobin and red blood cell values were similar in birds fed the control and 1g/kg MFeed® supplemented diets, packed cell volume and haemoglobin values were lower in these treatments than those in other dietary treatments. Lower values of white blood cells were recorded in all the treatments when compared with the starter phase; these values were still higher in birds fed the control and 1g/kg MFeed® diets. However the values obtained for creatinine, uric acid, alanine transaminase and aspartate transaminase decreased ($P<0.05$) as the level of MFeed® inclusion increased in the diets. All serum parameters measured with the exception of globulin were significantly ($P<0.05$) affected by the dietary treatment. Total protein values of birds fed control diet was significantly ($P<0.05$) lower than those in other dietary treatments. Alanine and aspartate aminotransferase decreased with increased in the level of MFeed® in the diets.

Table 1: Determined composition of basal diet fed to broiler chickens

Parameters	Starter phase	Finisher phase
Crude protein (%)	23.64	19.50
Fat (%)	10.12	10.05
Crude Fibre (%)	9.50	10.55
Calcium (%)	1.5	1.05
Phosphorus (%)	0.55	0.45
Energy (MJ/kg)	11.72	12.13

Table 2: Growth performance of starter broiler chickens fed Mfeed® supplemented diets

Parameter	level of Inclusion of Mfeed® in the diets (g/kg)					SEM	P-value			
	0	1	2	3	4		L	Q	C	
Starter (0-4 weeks)										
Average initial weight (g/bird)	43.21	43.19	43.28	43.23	43.10	0.09	0.679	0.625	0.810	
Average final weight (g/bird)	809.29	810.46	824.99	837.57	837.01	3.62	0.001	0.000	0.000	
Average weight gain (g/bird)	766.08	767.27	781.71	794.34	793.91	3.62	0.001	0.000	0.000	
Average feed intake (g/bird)	1285.06	1289.25	1241.10	1239.67	1249.71	10.83	0.995	0.057	0.132	
Feed conversion ratio	1.68	1.68	1.61	1.56	1.57	0.02	0.336	0.009	0.029	
Mortality (%)	5.56	5.56	2.78	0.00	0.00	1.29	0.018	0.067	0.119	
Duodenum pH	6.10	5.90	5.60	5.50	5.40	0.10	0.000	0.000	0.000	
Jejunum pH	5.80	5.70	5.50	5.40	5.20	0.10	0.000	0.000	0.000	
Finisher (5-8 weeks)										
Average Initial weight (g)	809.29	810.46	824.99	837.57	837.01	3.62	0.001	0.000	0.000	
Average Final weight (g)	1990.01	1989.56	2091.17	2234.19	2324.50	41.25	0.000	0.000	0.000	
Average Weight gain (g)	1180.72	1179.10	1266.18	1396.62	1487.50	38.08	0.000	0.000	0.000	
Feed Intake (g)	2678.21	2588.94	2386.69	2339.98	2367.18	42.27	0.031	0.000	0.000	
Feed conversion ratio	2.27	2.20	1.89	1.68	1.59	0.05	0.000	0.000	0.000	
Mortality (%)	5.88	5.88	0.00	0.00	0.00	0.10	0.123	0.289	0.449	
Duodenum pH	6.20	6.10	6.00	5.80	5.80	0.10	0.000	0.001	0.005	
Jejunum pH	6.30	6.30	6.10	6.00	5.80	0.10	0.000	0.001	0.003	

Table 3: Haematological parameters of finisher broiler chickens fed Mfeed® supplemented diets

Parameter	level of Inclusion of Mfeed® in the diets (g/kg)					P-value				
	0	1	2	3	4	SEM	L	Q	C	
Starter (0-4 weeks)										
Packed cell volume (%)	27.89	27.64	28.32	29.53	29.22	0.24	0.166	0.186	0.287	
Haemoglobin (g/dl)	9.18	9.33	10.42	10.63	11.22	0.21	0.000	0.001	0.002	
White blood cell (Cumm ³)	31,920	31,400	22,750	21,516	21,033	1,293	0.000	0.000	0.000	
Red blood cell (X10 ^{12/l})	2.39	2.37	2.65	2.77	2.72	0.07	0.017	0.000	0.000	
Finisher (5-8 weeks)										
Packed cell volume (%)	28.18	28.40	30.37	31.50	33.35	0.55	0.000	0.000	0.000	
Haemoglobin (g/dl)	10.34	10.45	10.94	11.03	11.25	0.19	0.000	0.000	0.000	
White blood cell (Cumm ³)	31,580	31,100	21,800	19,333	17,566	1,572	0.000	0.000	0.000	
Red blood cell (X10 ^{12/l})	3.23	3.29	3.39	3.40	3.69	0.06	0.006	0.025	0.070	

Table 4: Serum parameters of finisher broiler chickens fed Mfeed® supplemented diets

Parameter	level of Inclusion of Mfeed® in the diets (g/kg)					P-value				
	0	1	2	3	4	SEM	L	Q	C	
Starter (0-4 weeks)										
Total protein (g/l)	39.25	39.46	42.18	42.32	41.42	0.41	0.046	0.024	0.004	
Albumin (g/l)	23.95	23.81	25.99	26.46	24.59	0.44	0.289	0.230	0.056	
Globulin (g/l)	15.30	15.86	16.00	16.04	16.40	0.13	0.180	0.387	0.582	
Creatinine (g/dl)	0.91	0.91	0.89	0.89	0.87	0.00	0.007	0.001	0.003	
Uric acid (mg/dl)	1.43	1.42	1.40	1.37	1.36	0.01	0.020	0.020	0.039	
Alanine aminotransferase (iu/l)	87.82	79.54	74.49	70.83	70.26	0.51	0.000	0.000	0.000	
Aspartate aminotransferase (iu/l)	29.85	27.45	24.42	20.48	21.45	2.13	0.001	0.002	0.007	
Finisher (5-8 weeks)										
Total protein (g/l)	60.82	60.82	67.95	66.90	67.97	0.25	0.008	0.002	0.000	
Albumin (g/l)	23.95	23.95	38.38	39.90	41.75	2.45	0.000	0.000	0.000	
Globulin (g/l)	36.87	36.87	29.57	27.00	26.22	1.66	0.685	0.624	0.013	
Creatinine (g/dl)	0.99	0.99	0.97	0.95	0.95	0.02	0.010	0.001	0.003	
Uric acid (mg/dl)	7.64	7.64	6.85	6.20	6.36	0.19	0.002	0.002	0.005	
Alanine aminotransferase (iu/l)	90.75	90.75	88.43	80.26	80.82	0.94	0.000	0.000	0.000	
Aspartate aminotransferase (iu/l)	66.35	66.35	60.12	58.25	58.84	1.49	0.000	0.000	0.000	

Discussion

The improvement in body weight gain in broiler chickens fed MFeed® in the diets might be attributed to the unique quantum size effects of nanoparticles, lower intestinal pH values and acidic environment which makes more nutrients available to the birds for better performance. Andi et al. (2011) recorded a significant improvement in the weight gain, feed intake and feed conversion ratio of broilers fed Nanosil (silver nanoparticles). Pathogens are reported to grow in a pH close to 7 or slightly higher while beneficial micro organisms live in a most acidic pH (5.8-6.2) and compete with pathogens. Lowering of the duodenum and jejunum pH improved the digestion in broilers fed MFeed® diets which resulted in better growth. The pH level in the GIT had been reported to establish specific microbial population and also affects the digestibility and absorptive value of most nutrients. Boling et al. (2001) reported that lowering the pH by organic acids inclusion in diets improves nutrient absorption.

The catalytic action of MFeed® to boost enzyme activity of gastrointestinal secretions for better digestion and assimilation of nutrients could have resulted in the better utilization of feed thereby reduced feed intake of broilers fed supplemented diets. The combination of diatomaceous earth and montmorillonite may therefore improve the general health and indirectly boost their resistance to parasite. Montmorillonite clay has a high water absorption capacity and high cation exchange capacity, when added to feed is thought to have the additional benefits of slowing feed passage and improving feed efficiency (Biomin, 2007). Montmorillonite has been reported to be effective in treatment of irritable bowel syndrome (Ducrottee et al., 2005). Addition of diatomaceous earth to the diet of production animals has been used to control external and internal parasites (Macy, 2000). Mathis and McDougald (1995) found that feeding diatomaceous earth significantly improved feed conversion in broilers. The use of absorbent clay supplements has been shown to have some benefits in poultry by improving

feed efficiency; these compounds increase the absorption of nutrients by slowing gastric passage (Mallet et al., 2005). Seaweed have been shown to have prebiotic activity that stimulate growth and activity of beneficial microorganism in the digestive tract (El-Deek and Brikka, 2009)

The relatively lower white blood cell values obtained from bird fed MFeed® in their diets is an indication of better health condition or a lowered disease threat. These results are consistent with those obtained by Silversides et al. (2006) who reported increasing protection against toxins produced by pathogenic microorganisms using yeast extracts. Series of tests had reported the non-genotoxic effect of montmorillonite (Li et al., 2010; Sharma et al., 2010). Sultan et al. (2008) revealed a significant increase of 22.5 and 22.3% of haemoglobin and packed cell volume respectively in broiler chickens fed 0.5 g/kg in anise supplemented feed. A 28 week study in rats indicated that levels of calcium montmorillonite as high as 2.0% do not result in overt toxicity considering haematological parameters or clinical chemistry (Afriyie-Gyawu et al., 2005). Creatinine, uric acid, alanine transaminase and aspartate transaminase in the serum of broiler chickens improved with MFeed in the diets. These are indications that the liver and kidney were not negatively affected. Lovland et al. (2004) and Dahiya et al. (2006) reported that when chitosan is changed into nanoparticles, more amino groups and hydroxyl functional groups are exposed, and more immunoglobulins were induced by CNP-Cu. Serum immunoglobulins can further activate complements, enhance the immune system, and protect the birds from many diseases. This is contrary to works of Safaeikatouli et al. (2010) who reported that silicate mineral had no adverse effect on the blood serum parameters in broiler chickens. Also Drake and Hazelwood (2005) reported negative effects of silver nano-particles on serum uric acid but no effects on SGOT and SGPT. However, Sawosz et al. (2009) showed that nanoparticles had no effect on activity of enzymes; ASP, ALT and AP, and on concentrations of glucose, TG and cholesterol.

Conclusion

The results of this study showed improvement in body weight gain, feed conversion ratio and mortality when MFeed® was added to the diets of broiler chickens at 3g/kg and 4g/kg. However, 3g/kg inclusion as in-feed additive is recommended for economic reasons.

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IMPACT OF EMERGING ZONOSIS AND PUBLIC HEALTH SIGNIFICANCE IN NIGERIA

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Abstract

Emerging zoonotic diseases have assumed an increasing importance as a result of its negative impacts on the local, national and international human and economic policies in public and animal health care delivery services. Rapid detection of emerging diseases as well as precise effective actions against these diseases is slow in many developing countries. Morbidity and mortality due to zoonosis are mostly recorded due to deficiencies in the veterinary infrastructure, expertise, diagnostic laboratories and in surveillance capabilities. This review discusses updates on global impact of zoonosis, an evaluation of major zoonotic agents with particular reference to situation in Nigeria considering the effects of the farming systems of sub-Saharan Africa, as well as the attitudes and activities of the people as reported in the literature were also discussed. This review was carried out using internet search machines to evaluate the current situation through a review of data and publications devoted to the global control of zoonosis. Contributing factors that continue to affect emerging and re-emerging zoonosis in Nigeria include social, political and economic factors. Since zoonotic diseases are peculiar, the preparedness and response capability of a country towards an emerging disease largely depend on the availability of such facilities, therefore, their prevention and control will require unique strategies, based more on fundamental research than on traditional approaches thus necessitating a collaborative approach of local, national and international agencies working in human, animal health, food and other health practitioner communities are warranted, along with improved education of the general public and policy makers.

Key words: Emerging, zoonoses, factors, Nigeria, public health

IMPACT DES ZONOSES EMERGEANTES ET LEUR IMPORTANCE POUR LA SANTE PUBLIQUE AU NIGERIA

Résumé

Les zoonoses émergentes prennent une importance croissante en raison de leurs impacts négatifs sur les politiques humaines et économiques locales, nationales et internationales en rapport avec la prestation de services de soins de santé publique et animale. Les pays en développement affichent une certaine lenteur en ce qui concerne la détection rapide des maladies émergentes et la mise en œuvre de mesures efficaces et précises de lutte contre ces maladies. La morbidité et la mortalité dues aux zoonoses sont en grande partie imputables au manque d'infrastructures vétérinaires, d'expertise, de laboratoires de diagnostic et de capacités de surveillance. La présente étude passe en revue les informations actualisées sur l'impact mondial des zoonoses, une évaluation des principaux agents zoonotiques avec une référence particulière à la situation qui prévaut au Nigeria en prenant en considération les effets des systèmes agricoles de l'Afrique subsaharienne, ainsi que les attitudes et activités des populations tel que rapportées dans la littérature. L'étude a été réalisée en utilisant les machines de recherche sur Internet en vue d'évaluer la situation actuelle à travers un examen des données et publications consacrées à la lutte mondiale contre les zoonoses. Les facteurs qui continuent d'affecter les zoonoses émergentes et réémergentes au

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Nigeria comprennent les facteurs sociaux, politiques et économiques. Puisque les zoonoses sont d'une nature particulière, la capacité de préparation et de réponse d'un pays face aux maladies émergentes dépend largement de la disponibilité de ces infrastructures. Par conséquent, leur prévention et contrôle nécessiteront des stratégies uniques, basées sur une recherche plus fondamentale plutôt que sur les approches traditionnelles. Il sera nécessaire de mettre en oeuvre une approche collaborative des organismes locaux, nationaux et internationaux travaillant dans le secteur de la santé humaine, de la santé animale et de l'alimentation et d'autres communautés de praticiens sanitaires, avec une meilleure éducation du grand public et des responsables de l'élaboration des politiques.

Mots-clés : émergeant ; zoonoses ; facteurs ; Nigeria ; santé publique

Introduction

Zoonosis refers to any disease agent that moves into humans from an animal source (Willeberg *et al.*, 2011). Emerging diseases are a significant public health concern and cause considerable socioeconomic problems globally. An estimated report from developed countries stated that up to one third of the population are affected by microbiological food-borne diseases each year in which the significant disease burdens are now considered to be zoonotic (OIE, 2011). In Nigeria, no accurate data exist on disease episodes largely due to poor technology and disease reporting system (Oluwagbemi and Achimugu, 2010). The factors contributing to emerging and re-emerging zoonoses are complex and include social, political, environmental factors, microbial evolution and population growth. Environmental factors are external factors, such population growth, mobility and urbanization and environmental activities such as agriculture and livestock production, deforestation, and climate change. Presence and interaction of the above factors as well as the social and political activities, all imply that emerging zoonotic diseases will be of higher magnitude in proportion including their negative effects on health, animal health and food production (OIE, 2010).

Since about 75% of all emerging infectious diseases that affect humans originate from animals and 60% of all existing human infectious diseases are zoonotic with probable higher proportion in Nigeria as a developing country, in response to the teeming health challenges in 2009, the Nigeria Field Epidemiology Laboratory Training Program

(N-FELTP) was established in 2008 and has since strengthened the public health workforce in Nigeria. With support from WHO and CDC, N-FELTP trains field public health laboratory, epidemiology, and Veterinary epidemiology residents that worked in leadership and technical positions in the Nigeria Federal Ministry of Health (FMOH) and Agriculture and Rural Development (FMARD) in addition to leadership and technical positions at the state level.

The multidisciplinary collaboration has also addressed the existing and emerging infectious disease threats to animal and human health, food security, and global health significantly. The critical mass of epidemiologists required for optimum operation on zoonotic and multi-disease surveillance systems in Nigeria has not yet been fully realized. It is envisaged that as this initiative has produced a foundational cadre of midlevel public health practitioners with core skills in integrated disease surveillance, outbreak investigation, and response for zoonotic and other communicable and non-communicable diseases. It is also important that our health sectors will be identifying ways to successfully scale up and transform this innovative donor-driven program into a sustainable multi-sectorial one health workforce capacity development model by integrating it into our primary health care system (Becker *et al.*, 2012). The revised International Health Regulations (IHRs) are legally binding regulations that aim to encourage countries to investigate and report disease outbreaks and events that are considered to be public health events of international concern. IHRs exist as the primary legislative instrument to prevent the spread of infectious diseases

and minimize disruptions to international traffic and trade. Nigeria as a country has a lot of health challenges that are multifactorial but will hope to act toward IHR compliance in the nearest future for better assessment of their surveillance and response capacities and the development and implementation of plans of action to ensure that core functioning zoonotic public health capacities are sustained (Fasina *et al.*, 2008).

Definitions and scopes of emerging zoonosis

Infectious diseases are the world's leading cause of death for children and adolescents. They are the second leading overall cause of death after heart disease (UNEP, 2014). Muhie and Keskes (2014) in their review stated that "new, re-emerging or drug-resistant infections whose incidence has been increased within the past two decades or whose incidence threatens to increase in the near future" were defined as "Emerging Infections". A re-emerging disease is considered an already known disease that either shifts its geographical setting or expands its host range, or significantly increases its prevalence. Zoonoses are defined as diseases and infections which are naturally transmitted between vertebrate animals and man, but the emergence of zoonotic diseases caused by totally new or partially new agents led to diseases regarded as emerging and re-emerging zoonoses (Muhie and Keskes, 2014). Environments where wild animals, domestic animals and humans live in close proximity is a well-known factor which favors the emergence of new or already known diseases (Elwing, 2013).

Factors Contributing to the Emergence of Zoonotic Diseases

The factors contributing to emerging and re-emerging zoonoses are complex and include social, political, environmental factors, population growth and microbial evolution such as is the intrinsic factor that leads to the emergence of new pathogens. These important factors are discussed below.

1. Social factors

Various societal decisions and actions,

or lack thereof, can directly or indirectly cause emerging infectious diseases (EID) to be on the increase, causing various forms of harm to both human and environmental health. These social factors include increasing demand for animal protein, leading to changes in farming practices (example; large "open" poultry production units in Nigeria), animal markets, bush meat consumption, global trade, natural animal habitats hunt (example encroachment on forests), human behavioural changes that reflect ownership and movement of pets, ecotourism, boating, camping, food preferences (wild animals and raw milk), demographics (producing older and more susceptible populations), level of compliance with recommended prevention measures. The 2014 Ebola virus disease outbreak was largely attributed to contact between man and infected animals. The patient zero a two year old Emile Ouamouno, lived in the picturesque forest village (Medical express, 2014), such proximity to the forest as well as social behaviours could have been a major factor in the emergence of latest Ebola virus disease outbreak. Community participation is a critical factor in the control or total elimination of re-emerging infectious disease (Ngutor-Karshima, 2012).

2. Political factors

The surveillance and control of zoonoses depend upon the policies and action implemented at a local, national, regional and world level (Ngutor-Karshima, 2012). Government public health access and allocation of resources, including access to prevention programs, prophylaxis, and post-exposure treatment interventions contributes to the emergence of zoonotic diseases in Nigeria. Additionally, international political factors can have an impact, including limited or non-existent educational programs to support detection, identification and verification, and response, as well as limited or non-existent information technology and telecommunications infrastructure to establish surveillance links with high-risk areas of the globe. Inadequacy of the policies and actions implemented at just one of these levels can compromise the effectiveness of results achieved at the other

levels, regardless of their relevance or quality (Medical express, 2014).

3. Environmental factors

Environmental factors are external factors, environmental changes as a result of agricultural activities, deforestation, and climate change and environmental determinants such as ecologic and climatologic influences. One of the major goals of environmental regulation from the inception has been to reduce water pollution, there have been no clearly established, coordinated policy framework and standards for attaining such goal especially through resource pricing, incentives and taxes. Rather, heavy reliance has been placed on qualitative legal rules such as paying fines into local Government council officers. However, the benefits of clean environment would be available only if the generators of pollutants are encouraged to invest in pollution prevention and abatement technologies with the help of a judicious mix of regulatory policies, economic incentives and fiscal instruments.

4. Population growth:

As the human population doubles every few decades, so its impact on the environment increases as Humans cannot escape the law of population growth; when population increases, the resources required for its sustenance also increases, therefore man has made a remarkable impact on the environment primarily through the use of natural resources and production of wastes. Rapid human population growth has been identified as underlying environmental problems in the country population as well as strategies to reduce the negative impact of human activities on the environmental degradation (Iwejingi, 2012). Nigeria, which is referred to as the demographic Africa has been growing in such a way that the population figure recorded in every census surpasses the immediate past census. With a total population of over 160million people (FAO, 2012) and a growth rate of 3.2%, the nations will double its size in 22 years. This dramatic growth in human population has caused much concern especially about the strains it places on the resources of the environment and the quality of our lives.

5. Microbial evolution

Zoonotic disease-causing agents (Viral, bacterial or parasitic) bio-transforms for the purpose of adaptation to new vectors and hosts, undergo mutation and recombination/ re-assortment in humans and animals after exposure to multiple pathogens (food borne viruses, influenza viruses) resulting in the development of sustained increased virulence and drug resistance.

Emergence of new zoonotic pathogens seems to be increasing for several reasons: The global human and animal populations have continued to grow, global trade enhance fast distribution of infectious agents, ecologic and environmental changes brought about by human activity and bioterrorist activities are increasing, and in most instances the infectious agents implicated could be zoonotic (Murphy, 1998).

Specific major Nigerian Zoonosis

With a population of over 160 million and the need for improved health care delivery, Nigerians are at considerable risk considering the seriousness of these infections. Zoonotic infections that are endemic in Nigeria include; toxoplasmosis, rabies and Lassa fever. Zoonotic food-borne infections (caused by *Campylobacter*, bovine tuberculosis, Salmonellosis, *Escherichia coli* O157:H7) and cryptosporidiosis are emerging.

Rabies

Rabies is a viral disease caused by a rabid Virus of the family (rhabdoviridae) which is a major viral disease of the central nervous system that is enzootic worldwide and remains a serious public health hazard in Nigeria and other developing countries. Domestic dogs are usually regarded as the vectors of rabies (Burgos-Caceres, 2011) in most developing countries due to the high occurrence of non-vaccination of domestic animals in such countries, while wildlife rabies is more likely to be acquired in developed countries (Jackson, 2011). In Nigeria, rabies is an endemic disease, with dogs been implicated as the principal animal responsible for the spread of the disease (Qasim *et al.*, 2010).

Effective control, prevention and eradication of rabies in man and animal can only be realized through immunization. Despite concerted efforts to develop and produce anti-rabies vaccines for protection of man and animal against rabies since 1919 in Nigeria, successful achievement of effective control and prevention of the disease through vaccination is greatly hampered by poor or lack of modern technology and facilities required for development, production and storage of safe and effective vaccines. A study from the North central of Nigeria that looked into Identification, analysis of dog use and management practices in relation to rabies control in Ilorin city and its environs, underscores the importance of the status of community hygiene and environmental health as a major contributor to rabies risk (Aiyedun and Olugasa, 2011). Dog trading, slaughtering and consumption has also been identified as a major role in the epidemiology of rabies from dogs to humans in Nigeria (Ehimiyein, 2014). The High cost of development and production, poor electric power supply, poor policy implementation by governments, poverty and gross lack of awareness are other major constraints encountered in rabies vaccinology in developing counties including Nigeria (Tekki *et al.*, 2013). Mass vaccinations of domestic dogs have been shown to effectively control canine rabies and hence human exposure to rabies, education of the public and better government policy on stray dog will benefit the vulnerable populace. Knowledge of dog population demography is essential for planning effective rabies vaccination programmes; however, such information is still rare for African domestic dog populations, particularly so in urban areas.

Dengue

Dengue virus, which is now the most important and fastest growing insect-borne viral infection in the world, is transmitted primarily by the *Aedes aegypti* mosquito. The mosquito vector that transmits dengue multiplies in any small pool of stagnant water, especially in discarded tires or other detritus that may result from life in overcrowded urban areas. The disease, also known as “break – bon fever”, produces severe headache and disabling

pain in muscle and joints. Amarasinghe *et al.* (2011) stated that “during 1960–2010, a total of 22 countries in Africa reported sporadic cases or outbreaks of dengue; 12 other countries in Africa reported dengue only in travellers”

With a population of over 160 million and the need for improved health care delivery, Nigerians are at considerable risk considering the seriousness of these infections. Ayukekbong stated in his study that in Nigeria “Two independent studies among febrile patients revealed dengue IgG seroprevalence of 73% and malaria prevalence of 80% in some areas of the country” (Ayukekbong, 2014). He further highlighted that the diagnosis of the dengue disease is complicated by the fact that, dengue fever mimics malaria fever and the prevalence of dengue-malaria co-infection is high and suggested the use of differential diagnosis (Ayukekbong, 2014).

Bovine tuberculosis in Nigeria

Nigeria is one of the African countries where bovine tuberculosis is wide spread in both cattle and humans, its poor control poses a serious health challenge in the country (Okeke *et al.*, 2014). Although, the current status on the actual prevalence rate of bovine tuberculosis at a national level is unknown but from the limited survey research which have been reported over the last 30 years in the country, the prevalence of bovine tuberculosis due to *M. bovis* ranges from 2.5% in 1976 to 14% in 2007 which shows that the prevalence of the disease has been on the increase. The isolation and identification of *Mycobacterium bovis* in fresh and sour milk as sold in local market, sputum and tissue samples from humans especially among Fulani herdsmen, abattoir and slaughter houses has been reported. There are therefore various empirical evidences showing that bovine tuberculosis occurs in cattle and humans in Nigeria. Okeke *et al.* (2014), reported prevalence of *Mycobacterium Tuberculosis* complex as 21.4% by Acid fast bacilli test and 16.7% by duplex PCR in a study in Jos, Nigeria, Ejeh *et al.* (2014) reported a detection rate of gross pathological lesions of bovine tuberculosis (BTB) of (4.04%) in 2012, they also noted that there was a gradual increase

in the prevalence of BTB from 2008 to 2012 in Makurdi, Nigeria. In Ogbomoso, Nigeria, the prevalence rates was 5.0% of *M. bovis* based on tuberculosis testing and 1.7% based on acid fast staining (Ejeh *et al.*, 2014a). A prevalence rate of 6.5% was obtained in a similar study in Otukpo, Nigeria (Ejeh *et al.*, 2014b). Factors which contribute to the prevalence of BTB in Nigeria include; Bovine tuberculosis control in policies in Nigeria, lack of adequate veterinary services, eating habit and living standard of families.

Brucellosis in Nigeria

Brucellosis is an infectious disease caused by the bacteria of the genus *Brucella*. These bacteria can cause disease in many different animals. Humans become infected by coming in contact with animals or animal products that are contaminated with these bacteria. Bovine brucellosis is a disease of economic and public health significance in sub-Saharan Africa. The disease is still one of world's most damaging contagious bacterial zoonotic diseases in ruminants. The World Health Organisation (WHO) estimates 500,000 new cases of human brucellosis every year (Pappas *et al.*, 2006). The disease is distributed worldwide but some countries have been able to eradicate brucellosis from their territories using elaborate brucellosis control and eradication programmes that have been targeted primarily at livestock (the main reservoir host for the disease). This has been achieved mainly by vaccination, test and slaughter, as well as by regular surveillance for early detection of the disease (OIE, 2008). Despite the level of knowledge on the epidemiology of bovine brucellosis, there has been limited success in controlling bovine brucellosis in sub-Saharan Africa (Cadmus *et al.*, 2009). Some of the problems associated with the surveillance and control of bovine brucellosis in sub-Saharan Africa include poor disease reporting, insufficient financial resources of governments (poor economic status of most countries in sub-Saharan Africa), as well as competing national health priorities, inadequate infrastructures and personnel, the commonly practised seasonal grazing or trans-human husbandry systems and communal

grazing, inadequate monitoring of the disease in wildlife and poor communication and education of stakeholders (Ibironke *et al.*, 2008). In Nigeria it is endemic in animal populations and common cause of economic loss and ill health among animals and occupationally exposed human populations. In a study, patients with pyrexia of unknown origin (PUO) who was predisposed to brucellosis through rearing of animals and consumption of different animal products were tested for presence of *Brucella abortus* antibodies using Rose Bengal and serum agglutination antigens. Twenty six (5.2 %) of the 500 patients had *B. abortus* antibody. The high titres of 320, 640 and 1280 obtained in the sera of patients in their study were suggestive of the endemicity of the disease in their study location (Baba *et al.*, 2001).

Toxoplasmosis:

Toxoplasmosis is one of the most common zoonoses worldwide. *Toxoplasma gondii* is a protozoan parasite that infects most species of warm blooded animals, including humans, and can cause the disease toxoplasmosis. The only known definitive hosts for *Toxoplasma gondii* are members of family Felidae (domestic cats and their relatives). Unsporulated oocysts are shed in the cat's feces which are usually only shed for 1-2 weeks. Oocysts take 1-5 days to sporulate in the environment and become infective. Symptoms include cramps abdominal pain, fever, nausea, and vomiting associated with a marked weight loss. Humans can become infected by eating undercooked meat (Boughattas *et al.*, 2014) of animals harbouring tissue cysts, consuming food or water contaminated with cat feces or by contaminated environmental samples (such as faecal-contaminated soil or changing the litter box of a pet transplacentally from mother to foetus. Particular risk factors are characterized by immunosuppression and pregnancy. Congenital toxoplasmosis associated with miscarriage, hydrocephalus, cerebral calcification and chorioretinitis in the newborn (Flatt and Shetty, 2013). A study in Nigeria reported that 29% of 374 pregnant women in Zaria were seropositive for IgG and

0.8% to IgM (Ishaku, 2009), in their findings, the habit of eating undercooked meat was directly correlated with higher prevalence. In a survey from Maiduguri-North eastern Nigeria, 36.2% (38 of 105 cats) were seropositive for *T. gondii* (Kamani *et al.*, 2010). In their study, larger proportions of cats (56%) are stray cats in our environment than owned thereby increasing their chances of shedding oocysts in the environment. These potential routes of infection are important as critical points that could be targeted in strategic plan to try to reduce risks of toxoplasmosis in a susceptible population (Mandell *et al.*, 2005).

The best approach to minimizing the risk of contracting toxoplasmosis is by avoiding direct contact with cat faeces, wash hands after handling cats or cat equipment, wear mask and gloves when handling manure or for any soil contact and cook all meat properly. Congenital toxoplasmosis can be prevented through screening of *T. gondii* infections during antenatal care (Mwambe *et al.*, 2013).

Campylobacter in Nigeria

Campylobacteriosis is a collective description for infectious diseases caused by members of the bacterial genus *Campylobacter*. In developing countries, *Campylobacter* is the most commonly isolated bacterial pathogen from children less than two years old with diarrhoea. Infection occurs in adults and older children in developed countries. Gwimi *et al.* (2015), confirmed the presence of *Campylobacter* species in pigs and human fecal samples obtained in Kebbi state Nigeria. Most estimates of incidence in developing countries are from laboratory-based surveillance of pathogens responsible for diarrhoea. *Campylobacter* isolation rates in developing countries range from 5 to 20 percent (Oberhelman and Taylor, 2000). Community-based case studies have provided estimates of 40 000 to 60 000 cases for every 100 000 children younger than five (Rao *et al.*, 2001). In Nigeria, gastroenteritis due to *C. jejuni* was first reported in northern part of the country in 1981 and the South-Western part in Ile-Ife in 1983 (Aboderin *et al.*, 2002). In a prospective case control study that was carried out at University of Ilorin Teaching

Hospital (UIITH), private hospitals and primary health centers in Nigeria, three hundred and six (306) children with diarrhoea and 100 without diarrhoea were randomly selected. Isolation of *Campylobacter jejuni/coli* from stool samples revealed that twenty five (8.2%) of the patients and none of the controls had the organism accounting for *Campylobacter jejuni* and *C. coli*; 56% and 44% respectively (Samuel *et al.*, 2006). In a similar study, 303 children with diarrhoea and 100 children with other medical conditions other than diarrhoea were randomly selected. Fifty eight (19.1%) and 6% of the patients and controls, had the organism respectively. *Campylobacter coli* accounts for 53.3% of isolates (Coker *et al.*, 2002). They both concluded that *Campylobacter jejuni* is an important diarrhoea agent in our environment and recommended a complete characterization of local strains (Samuel *et al.*, 2006). In addition, national surveillance programs and international collaborations are needed to address the substantial gaps in the knowledge about the epidemiology of campylobacteriosis in developing countries (Wesley and Miller, 2010). Arcobacters are aerotolerant *Campylobacter*s. Arcobacters are emerging zoonotic enteropathogens Arcobacter species have been discovered as both animal and human pathogens within the past two decade (Snelling *et al.*, 2006). The genus Arcobacter has become increasingly important in recent years because its members have been considered emergent enteropathogens and potential zoonotic agents (Vandenberg *et al.*, 2004). The clinical features of the bacterial infection in humans is dominated by gastroenteritis and occasionally, extra intestinal manifestation; septicaemia, endocarditis, arthritis, peritonitis, liver cirrhosis while some Arcobacter species have been detected in or isolated from stools of patients with and without diarrhoea (Cardoen *et al.*, 2009). Increase in isolation from clinical, food and animals sources have led to its been classified as a serious hazard to human health by the International Commission on Microbiological Specifications for Foods (ICMSF, 2002) and recently as a significant zoonotic pathogen (Amisu *et al.*, 2002). Arcobacter was first isolated in Nigeria in Lagos metropolis

between 1999 and 2001 (Adesiji, 2010) where prevalence rate of 17% of *Arcobacter* was detected from abattoir chicken effluents and subsequently from chicken and pigs (Adesiji *et al.*, 2011). In order to reduce the risk represented by zoonotic agents to the consumer health, it is essential to reduce contamination of carcasses during the slaughtering processes. Therefore the maintenance of slaughter hygiene and marketing condition to retain keeping quality is consequently of central importance in meat production.

Salmonellosis in Nigeria

Food borne outbreaks caused by *Salmonella* represent an important public health problem worldwide (Raufu *et al.*, 2014). An estimated 1.4 million cases of salmonellosis, leading to 16,000 hospitalizations and nearly 600 deaths, occur each year in the United States. *Salmonella* infections in humans often result from the ingestion of contaminated foods, such as poultry, beef, pork, eggs, milk, seafood, and fresh produce (Gorman and Adley, 2004). Food contamination with antibiotic-resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections (Adesiji *et al.*, 2014). In developing countries poor sanitary conditions appear to be the main risk factor for the transmission of *Salmonella* spp. *Salmonella* enteric serovar Typhimurium (group D) and *S. enterica* serovar Enteritidis (group B) have been reported to account for 79 – 95% of all bacteraemic non-typhoidal *Salmonella* infections in sub-Saharan Africa and also foodborne outbreaks (Tennant *et al.*, 2010). In Nigeria, morbidity associated with illnesses due to *Salmonella* continues to be on the increase and, in some cases, resulting in death. Like other countries, treatment of patients has been based on the use of first-line antibiotics, such as chloramphenicol and co-trimoxazole, and the third-generation cephalosporins. Moreover, majority of Nigerians live below poverty level and patronize low cost foods such as those sold in “bukas”, however the hygiene conditions of those “bukas” in some cases

are not ideal. Active nationwide surveillance on molecular epidemiology of salmonellosis is non-existent in Nigeria. However a study that examined molecular characterisation of *Salmonella* isolates from raw foods and foods sold by food handlers in Lagos Nigeria and compared the isolates with clinical samples of *Salmonella* spp from patients presenting with febrile illness confirmed positive association of clonal fingerprinting (Smith *et al.*, 2011). Their study confirmed the significance of food selling points in transmission of *Salmonella* infection in Nigeria. In developing countries like Nigeria, reducing the incidence of salmonellosis especially in children requires the provision of safe drinking water, observance of good personal hygiene by mothers, hygienic food preparation and effective sewage disposal. Furthermore, the provision of adequate health care, wholesome water for drinking and domestic use by governments cannot be overemphasized (Adejuyigbe *et al.*, 2004).

Escherichia coli O157:H7

E. coli O157:H7 is the predominant serotype of *E. coli* that form one group of EEC. This EEC group is termed enterohemorrhagic *E. coli* or EHEC. *Escherichia coli* O157:H7 is an enterohaemorrhagic strain of the bacterium. *Escherichia coli* and *Salmonella* spp food-borne diseases outbreak impose a substantial burden on health care systems and can markedly reduce the economic productivity of the countries (Akbar *et al.*, 2014). Most of the infections caused by the bacteria are due to two Shiga toxins, termed Stx 1 and Stx 2 and also termed Vero toxins. These toxins are almost identical to toxins produced by another related bacterium, *Shigella* spp that causes dysentery and can damage and kill intestinal cells and occasionally cause anaemia, damage to platelets, and death of cells in other organs, especially the kidneys (Paton and Paton, 1998). *Escherichia coli* O157:H7 have been implicated in severe human diseases, including bloody diarrhoea (haemorrhagic colitis) and haemolytic uremic syndrome (HUS) (Coia, 1998) which occasionally leads to kidney failure especially in young children and elderly people. Cattle faeces have been recognised as the principal

reservoir of the microorganism in waterborne and food-borne *E. coli* O157:H7 outbreaks and sporadic infections (Armstrong *et al.*, 1996 and Coia, 1998). Farm animals are asymptomatic carriers of STEC and contaminated meat is an important vehicle for zoonotic transmission from animals to humans. A study investigated the presence, virulence traits and antimicrobial susceptibility of seven potentially human pathogenic STEC serogroups in the faeces and meat of food-producing animals in Ibadan, Nigeria with a prevalence of 46- 49.4%. One hundred and fifty-four (7.3%) of 2133 samples were positive for STEC serogroups (Ojo *et al.*, 2010). Their study emphasizes the risk of *E. coli* O157: H7 along the meat chain and the need for concerted effort to limit it through best hygiene practices. A study that evaluated retailed beef and related meat products for *E. coli* O157 in Zaria, Nigeria reported a prevalence of 2.2% and also in their study they implicated poor sanitary environment under which animals are slaughtered, transported, processed and sold (Tafida *et al.*, 2014).

Approach to sustainable control

In today's globalized world, diseases have the potential to transcend geopolitical boundaries through international travel and trade. It is now understood that the economies and livelihoods of the entire international community can be affected by a single health crisis in one. In today's globalized world, diseases have the potential to transcend geopolitical boundaries through international travel and trade. It is now understood that the economies and livelihoods of the entire international community can be affected by a single health crisis in one country. With this concept in mind, a revised version of the World Health Organization (WHO), International Health Regulations (IHRs) was established in 2005 (effective 15 June 2007) to "help the international community prevent and respond to acute public health risks that have the potential to cross borders and threaten people worldwide" (OIE, 2011). This binding legal instrument covers 194 countries across the globe, including all WHO Member States, and aims to protect public health through the

prevention of the spread of diseases for that purpose and as part of IHR implementation, WHO Member States are committed to strengthening their surveillance of and ability to rapidly detect, assess, notify, and report potential Public Health Emergencies of International Concern (PHEICs) in accordance with these regulations. Training professionals with a holistic vision of potential risk situations and the practical aptitude to coordinate actions with different sectors is vital. The veterinary medical discipline is one of the sciences that should consider reviewing their curriculum and accepting more responsibility related to protection of human health (Becker, 2003).

Conclusions and recommendations

Salmonella, *Campylobacter* and *E. coli* O157:H7 contribute most significantly to the burden of food borne bacterial zoonotic illness across both the developed and developing world. However, there are a number of emergent species, serovars and clones of bacteria which also impact on the disease burden. These include *Enterobacter sakazakii*, emergent species of *Campylobacter* and *Arcobacter*, new serovars of verocytotoxigenic *E. coli* and multi-antibiotic resistant bacteria. Bacteria will continue to evolve genetically and coupled with ongoing changes in the food sector; this will no doubt lead to the continued emergence of new food poisoning bacteria and new routes and vectors of transmission. The key to addressing and managing emergent zoonotic diseases and disease causing agents is the prompt action for early detection, diagnosis, treatment of patients with mild signs and symptoms and a quick intervention to prevent mass contact and spread of any diagnosed disease. The ability to track these organisms along the entire "farm to fork" chain is very important and should be given a first firm priority. This can give the food sector scientific evidence about where these food poisoning bacteria and other zoonotic disease causing agents are entering a process, where cross contamination may be occurring, whether a particular bacterial strain is endemic in a factory environment, and most importantly, where controls should be directed. In a food

poisoning outbreak the ability to track a bacterial isolate from a human patient back to a food or environmental source provides essential information about the cause of the outbreak and can give the scientific basis for a product recall. Equally in an age of global food trade the ability to track food poisoning bacteria across international borders using standardised protocols and databases is critical.

In conclusion, it is clear that better understanding of the occurrence of zoonoses and diseases common to man and animals in potential public health emergency of international concerns (PHEICs) will allow for more efficient allocation of resources, both financial and human, and better in country verification and response, supported by international organizations. Multidisciplinary teams including physicians, veterinarians, environmentalists, epidemiologists, communicators, and others are crucial to the success of the IHR implementation. Evidence-based analysis of the animal/ human health interface could also support new directions in research, vaccines, and treatments, as well as the development of diagnostic tests and other tools, to help prevent, control, eliminate, or mitigate emergent infectious diseases (FAO, 2008). The recommendations of the “One World, One Health” strategic framework—particularly the need to develop surveillance capacity, strengthen public and animal health capacity, strengthen national emergency response, and promote inter-agency and cross-sectorial collaboration—provide some helpful guidance for these types of endeavours (FAO, 2008). In Nigeria, The animal Health Decree Acts of 1978 should be revised to include all one health strategies and approach. All stake holders particularly the Ministry of Health, Ministry of Agriculture and rural Development, National Administration of Food and Drug Control, Ministry of environmental Health and Sanitation in Nigeria should embrace and evaluate strategies and logistics in practicability of One Health approach to facing the challenges of zoonotic menace.

Early detection of highly infectious pathogens responsible for epidemic or pandemic-prone diseases, or those that

otherwise pose a threat to world populations or economies should be given a local, national and global desired urgent attention to avert the catastrophic effects they may cause.

Timely and accurate verification of the presence or absence of these pathogens using diagnostic methods in the field, in laboratories, and in health care settings; and a comprehensive and rapid response to care for infected patients and reduce exposure of the wider population to contaminated food, infectious humans and animals, and the accidental and/or deliberate release of high-consequence pathogens should be handled appropriately with maximum urgency. Timely visit to hospitals for proper diagnosis of mild ailments and routine checking for determination of health status against especially zoonotic and communicable diseases and strategic deworming is hereby recommended.

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SUSTAINING N'DAMA CATTLE FOR THE RESOURCE-POOR FARMERS IN THE GAMBIA

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Abstract

N'Dama cattle which is endemic to West and Central African countries is a part of global livestock biodiversity that needs to be sustainably conserved in order not to lose its unique genetic characteristics which are important for meeting the challenges of insufficient animal protein production, food insecurity, rural poverty and climate change. This study assesses the genetic improvement, sustainable production, utilization and conservation of this breed of cattle in order to strengthen them through relevant technical strategies and policy measures. Review of relevant literature and policy documents, participatory group discussions were used while the information gathered was analysed through content analysis. Efforts to sustainably improve, utilize and conserve the adaptive traits of N'Dama cattle which are able to tolerate trypanosomosis and survive on low-quality feed will serve as impetus for the farmers. Particularly, strengthening of open nucleus breeding scheme, institutional support of the multipliers, financial and technical support of the extension services, and favourable policy environments are the packages that would maximize the potentials of N'Dama cattle in terms of food production and reliable income generation for the resource poor farmers in a country such as The Gambia.

Keywords: N'Dama; Production; Utilization; Conservation; Policy; Nucleus breeding.

PRÉSERVER LA RACE BOVINE N'DAMA POUR LES ÉLEVEURS DÉPOURVUS DE RESSOURCES EN GAMBIE

Résumé

La race bovine N'Dama, une race répandue dans les pays de l'Afrique de l'Ouest et de l'Afrique centrale, fait partie de la biodiversité bovine mondiale qui doit être durablement préservée afin de ne pas perdre ses importantes caractéristiques génétiques uniques qui peuvent permettre de relever les défis liés à l'insuffisance de la production de protéines animales, à l'insécurité alimentaire, à la pauvreté rurale et au changement climatique. La présente étude évalue l'amélioration génétique, la production, l'utilisation et la conservation durables de cette race de bovins, afin de les renforcer en utilisant des stratégies techniques pertinentes et des mesures politiques. L'étude a fait appel à un examen de la littérature et des documents politiques pertinents et aux discussions de groupe participatives ; et l'information recueillie a été analysée à travers l'analyse de contenu. Les efforts visant à améliorer, utiliser et conserver de manière durable les caractères adaptatifs des bovins N'Dama, qui sont trypano-tolérants et de survivre avec une alimentation de faible qualité, serviront d'encouragement pour les agriculteurs. En particulier, le renforcement du système de sélection à noyau ouvert, l'appui institutionnel des multiplicateurs, le soutien financier et technique des services de vulgarisation et la création d'environnements politiques favorables sont des programmes qui permettraient de maximiser les potentialités des bovins N'Dama en termes de production alimentaire et de génération de revenus fiables pour les agriculteurs dépourvus de ressources dans un pays comme la Gambie.

Mots-clés : N'Dama ; Production ; Utilisation ; Conservation ; Politique ; sélection à noyau ouvert

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Introduction

The present day farm animals through the domestication processes that occurred several thousand years ago originated from more than one wild ancestor while the highest level of diversity is observed among the animals with long history of domestication and development (Diamond, 2002; Freeman *et al.*, 2006). Interestingly, archaeological findings and genetic marker analyses of indigenous cattle from the African continent indicated that the present-day humpless cattle which include the N'Dama breed was the earliest with an history dated as far back as 8000BC (Gifford-Gonzalez and Hanotte, 2011).

Following its initial domestication, N'Dama cattle through pure and crossbreeding programmes have been utilized in various production environments to meet nutritional demand, cultural, agricultural, and socio-economic needs of the resource poor farmers. Importantly, this breed because of its unique characteristics had been crossed with the New Jersey, Red Poll, Sahiwal, Fulani Zebu, Sokoto Gudali, and Simmental (Felius, 1995; Meyn, 2005) in order to maximize its disease resistant trait and also to increase its production potentials in the low-input production environments. Those mentioned crossbreeding activities were carried out within the African countries with a possible indication that the N'Dama genes may not have spread widely outside this region. Also, N'Dama cattle is a transboundary regional breed i.e. found in Africa which is one of the seven regions (Asia, Europe and the Caucasus, Latin America and the Caribbean, the Near and Middle East, Africa, North America, and the Southwest Pacific) defined for the State of the World Animal Genetic Resources (FAO, 2007a). However, there are evidences that this breed which originated from the Fouta-Djallon highland of Guinea had spread either in the live form or through gene transfer to over 20 countries thereby making it the 20th most widely spread cattle breed in the world (FAO, 2009).

As an important part of the global biodiversity, the N'Dama cattle is highly endemic to West and Central Africa. Within

these sub-regions, N'Dama cattle is remarkably productive under the moderate to high tsetse fly challenge areas (Fall *et al.*, 2003). Meanwhile, the relative high number of N'Dama cattle in the developing countries like The Gambia, Guinea, southern Senegal, Guinea-Bissau, Mali, Liberia, Sierra Leone, Congo and Gabon is related to its distinguishable disease tolerant abilities, relatively high adaptability to prevailing local climatic conditions, conservative nature of the farmers, traditional and socioeconomic importance attached to the animals. The importance of this cattle breed to smallholder farmers was also emphasized in a survey carried out in The Gambia during which 97% of the respondents signified their preference for this particular breed to any other trypanotolerant breed like West African Shorthorn cattle for draught purposes (CR-Gambia, 2003). Furthermore, its characteristic resistance to parasitic infections and its ability to survive on low quality feed when compared to the Zebu breed are among the factors for its high preference by the smallholder farmers. Despite those favourable adaptive traits resulting in food and agricultural utility values of the N'Dama cattle, the production, productivity and especially, the competitiveness of this breed still faces challenges in terms of seasonal shortages of feed and water, absence of measures for controlled mating, and gradual loss of natural habitats. In addition, the typical low-input system of animal husbandry, increased demographic pressure on available grazing land, inadequate veterinary services are among the threats confronting the resource-poor farmers in maximizing the potential of N'Dama cattle for draught power and animal protein production in the form of milk and meat.

Meanwhile, by considering the multipurpose utility functions of the N'Dama cattle, there is a need to adequately conserve this endemic animal that are domicile in the West and Central African countries in order to prevent gradual loss of its desirable genetic traits. In this regard and within the scope of this study, the technical, institutional and political supports were regarded as important incentives that would encourage the resource-

poor farmers to continue the husbandry of this breed as a source of food, income, livelihood and climate change adaptability strategies. Therefore, this study using The Gambia as a case study constructively examines past and present efforts taken towards sustainable genetic improvement, production, utilization and conservation of the N'Dama cattle. The choice of The Gambia among other countries is based on the high percentage (>90%) of N'Dama breed in the national cattle herd, its high preference and affordability by the resource-poor cattle husbandry men. Also, this study through careful assessment of the relevant Gambian national policy and regulatory frameworks offers useful suggestions for sustaining the husbandry of N'Dama cattle by resource-poor farmers and other stakeholders.

Material and Methods

Participatory group discussion

In order to extract information that is relevant to answer the research questions, the scientific articles, magazines, reports, conference proceedings, and websites were reviewed. The facts gathered from those literatures were used to describe the breeding and conservation strategies for the N'Dama cattle. This review section provided the clues and basis for the information in the semi-structured questionnaire used for the participatory group discussion about the on-going pure N'Dama breeding scheme of the International Trypanotolerance Centre (ITC), The Gambia. Also, this approach was used to assess the awareness and knowledge level of farmers concerning the relevant policies that are meant to enhance the overall management and production of N'Dama cattle. Meanwhile, the first set of questions during those discussions assessed the demographic information of the participants after which it was duly followed by series of questions focusing on the participants' general knowledge of N'Dama breed and then the Open Nucleus Breeding Scheme (ONBS) of ITC, The Gambia. The initial set of questions on local protocols and regulations served as warm-up for the participants. This activity gradually progressed

into the discussions focused on the knowledge of the participants about relevant agricultural and natural resources (ANR) policies of The Gambia which highlighted the needed steps to achieve a sustainable management of animal genetic resources (AnGR) including the N'Dama cattle.

The group discussions held at Keneba, Niamina and Nianija averagely consisted of 11 participants that were selected based on their good understanding of the subject matter. The specific choice of those sites was based on the relatively high number of N'Dama cattle breed and the existence of an integrated livestock development project called PROGEBE (Regional Project on Sustainable Management of Endemic Ruminant Livestock in West Africa). Also, gender, cultural and social factors that are embedded in the interpersonal relationships of farmers were considered in selecting the participants. However, 95% of the eventual participants were men because cattle ownership in The Gambia is rather associated more with men than women. The discussions were conducted in the local languages (Fula, Madinka, and Wollof) of the participants through translation of the original English script by the moderator while script writing was done simultaneously by the researcher and the attached livestock assistant who understood that local language of the interviewed farmers' group. During the discussions, group dynamics, motivation and curiosity was ensured in order to gather sufficient information from the respondents. The information collected through participatory group discussions were complemented by interview of specific stakeholders which included livestock project coordinators, scientists, livestock technicians, veterinary officers, literate farmers, and policy makers. This process enhanced a deeper insight into the group discussions because it enhanced further clarity of some contextual statements.

Content analysis

The documents, relevant literatures and reports were reviewed by searching for keywords including N'Dama cattle, production, policy, conservation, utilization, nucleus breeding that were in the research objectives.

The paragraphs of the document that specifically contained those keywords were highlighted, contextually coded and reviewed in connection with the research objectives. Through this process, the initially voluminous qualitative data were reduced to readable and descriptive texts. The relevant information was also summarised in a tabular format. Various responses from the participatory group discussions were written in paragraphs and collated on the basis of assigned keywords. With due attention to patterns and trends, the coded sentences and paragraphs were clustered into specific categories that could answer the research questions (Weber, 1990). The outcomes of this procedure were the descriptive sentences and paragraphs that could answer the research questions. This method was used to detect the extent and trends of activities over a particular period of time. It also enhanced identification of certain core consistencies and meanings from a volume of qualitative materials (Patton, 2002).

Results

Breeding and improvement of N'Dama cattle

In order to sustain an adaptive utilization and conservation of N'Dama cattle by farmers, efforts were previously made to

genetically improve the desirable traits of this breed. Among the remarkable innovative steps taken was the design and implementation of a pure breeding programme called an Open Nucleus Breeding Scheme (ONBS). Concerning this ONBS that is operated by the International Trypanotolerance Centre (ITC), it represents a scheme where due priorities are given to phenotypic selection, screening, breeding and genetic improvement of endemic ruminant species. The pictorial description of ONBS for N'Dama cattle is as shown in Fig. 1 with the arrows pointing in the different directions through which transfer of animals are carried out within the three tiers of this scheme. An important benefit of this ONBS is that the genetic gain of the animals in the nucleus herd is permanent, cumulative and can be disseminated to other farmers' herds.

In the Gambia, the breeding scheme for N'Dama cattle started in 1994 with the objective of improving milk and meat while maintaining the unique characteristics of the breed in terms of trypanotolerant and adaptive traits. To achieve those objectives, the nucleus animals have always been systematically selected based on the estimation of their breeding values using the Best Linear Unbiased Prediction (BLUP) method. To simulate the

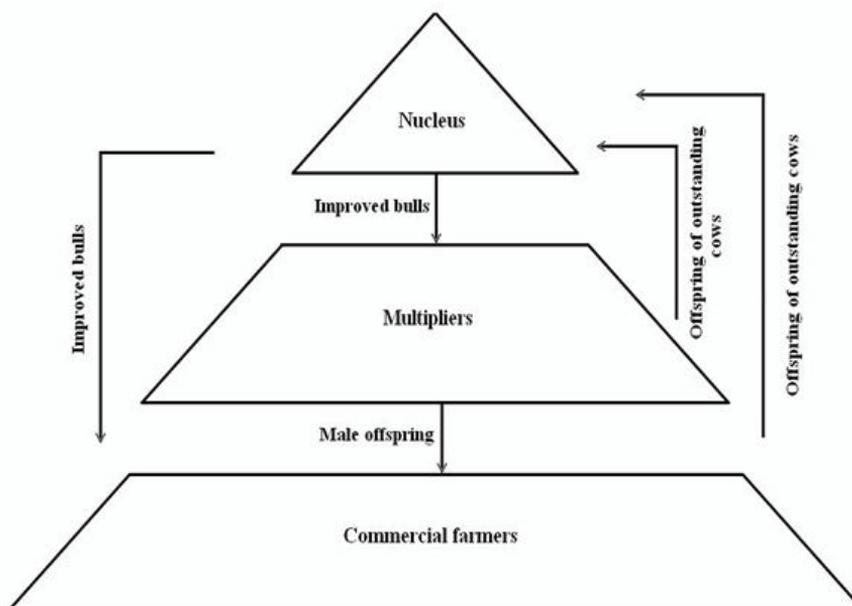


Figure 1: An Open Nucleus Breeding Scheme (Dempfle and Jaitner, 2000)

natural habitat of N'Dama cattle, the feeding and other management systems at the nucleus station are made similar as much as possible to the on-farm situations while sustainability and within-breed diversity in the ONBS is ensured through a mechanism that permits screening and introduction of outstanding offspring from farmers' herds into the nucleus. In addition, adequate involvement of multipliers and their association called Gambian Indigenous Livestock Multipliers' Association (GILMA) in the dissemination of improved bulls combined with training of livestock assistants, farmers and multipliers were planned and ensured until 2006. Unfortunately, trend of the described activities have changed due to limited funds, reduced number of nucleus animals and the collapse of multipliers' association. Also due to insufficient financial resources of ITC, and inadequate human capacity and extension services in the ONBS, the introduction of new animals from the outside herd into the nucleus has been suspended thereby resulting in a limited number of disseminated bulls and farmers' involvement in the whole breeding scheme. The following paragraphs therefore highlight some approaches that could be explored in order to solve the challenges of ensuring sustainable production and genetic improvement of the N'Dama cattle.

Sustaining the N'Dama cattle

Technical options

Given the challenges in meeting the farmers' needs for genetically improved elite bulls through ONBS, expanding the nucleus herd by introducing more bulls and increasing both the technical and financial resources were considered highly essential by the respondents in this study. However, this will require the commitments of government and other stakeholders that have interest in the sustainable utilization and conservation of endemic ruminant species. Such collaborative efforts in the form of a project (2008-2014) sponsored by the African Development Bank and the Global Environment Fund through the United Nations Development Programme which is favourably changing the landscape for

endemic ruminant animal genetic improvement and management in the selected four West African countries (Gambia, Guinea, Senegal and Mali) was emphasized. This project through provision of infrastructures and capacity building of farmers and other actors is stimulating interests in the production of the three trypanotolerant ruminant species (N'Dama cattle, West African Dwarf, and Djallonke sheep) and at the same time raising awareness about the need for their sustainable conservation.

As an alternative approach to the phenotypic selection in the ONBS, the more efficient technique called Marker Assisted Selection (MAS) and genomic selection can be explored because this will reduce generation interval and accelerate the rate of genetic gain in the ONBS. There is also a need to step up the initial approaches for dissemination the elite bulls by exploring new methods and latest advances in reproductive technology such as artificial insemination with the aim to fit this into the local context of farmers.

Concerning the dwindling institutional support for genetic improvement of N'Dama cattle breed, the International Trypanotolerance Centre which is regionally responsible for breeding of elite N'Dama bulls should be both financially and technically supported towards an enhanced fulfillment of its institutional mandates. Also, the Department of Livestock Service which is responsible for extension and training of livestock farmers should be duly supported in terms of human and technical capacity building. Empowerment of such institutions toward effective delivery of their mandates will motivate the farmers and other stakeholders thereby stimulating a multistakeholders' approach to the management of N'Dama cattle. Furthermore, organized marketing systems through private-public partnership and development of important marketing infrastructure will provide mediums for the farmer to easily link up with the potential input suppliers, livestock buyers and consumers. Training of farmers on alternative livestock feed production, processing, storage and supplementation will assist them in overcoming the challenge of

seasonal food shortage. Also, adequate access to veterinary services can be facilitated through training of more livestock technicians and veterinarians towards early animal disease detection, prevention and control strategies.

Relevant policies options

Contextually, there are policy frameworks in place to guide various actors in The Gambian livestock sector. Those policies were formulated on a periodical basis with each framework spanning from four to six years. The first of its kind is the framework called “The First Five Year Development Plan” that lasted from 1975 to 1980. Since then, other frameworks that recognised the important roles of endemic ruminant livestock in the production of animal protein and also in economic development through foreign exchange had been designed with the most recent one called the “Agriculture and Natural Resources (ANR) Policy 2009-2015”.

Specifically, analysis of the Gambian ANR Policy shows that improvement of livestock production and productivity to a level that surpasses any previous achievement is a key objective of the national government. Also included as the targets of this policy framework is the provision and enforcement of guidelines, regulations, and measures that will ensure a sustainable management of the country’s natural resource base. However, this policy that contains details of what the livestock sector should be in a future term is nonetheless silent on how sustainable conservation of animal genetic resources within the Gambian territory can be holistically achieved. To address this issue, the interviewed farmers and livestock production experts suggested that the government needs to involve livestock keepers and other actors along the livestock value chain in the review and design of an improved policy version in order to increase its recognition among these stakeholders. Also, proper and joint evaluation of N’Dama cattle in terms of their nutritional, cultural, economic, agricultural and environmental values should be prioritized in this exercise. This process will enhance effective policy dialogue among the relevant stakeholders and provide a platform

for a wide recognition and implementation.

Perspectives on the impacts of existing policies

The farmers in the participatory group discussion and who are also the main custodians of N’Dama cattle breed were not explicitly aware of any national policy that concerns the N’Dama cattle production or conservation. The reason for this remark can be attributed to high illiteracy level of the farmers which potentially impedes proper understanding and interpretation of relevant government initiatives. However, the combined approach to management of agricultural and natural resources which was the objective of The Gambian ANR policy was familiar to the interviewed farmers. Nevertheless, the farmers observed that the Gambian ANR policy have also restricted livestock herd movement as well as limited the size of available grazing area for the ruminant animals especially during the dry season. The Gambia National Agricultural Investment Plan (2011-2015) which was designed to provide opportunities for enhanced management of shared resources, pastoral infrastructure, control of transhumance, greater awareness and adherence to harmonized regulatory and health provisions is addressing some of these stakeholders’ concerns. In order to enhance awareness of ANR policy and other related protocols that entail sustainable production, utilization and conservation of N’Dama cattle, the farmers advocate for more representation and flexibility in the general policy making processes.

The review of The Gambian past and present AnGR related policies shows that N’Dama cattle is an essential asset to meet the national demand for milk, meat and draught power. As a result, increase in N’Dama cattle production has always been inherent objectives of different Gambian policy frameworks that target a sustainable AnGR management. However, some farmers and other stakeholders such as livestock technicians and scientists are not duly aware or appropriately informed of those policies. They claimed that policy documents are available to certain top government officials while accessibility to other field staff is occasionally restrained.

Table 1: Summary of the relevant Gambian policies in relation to production and utilization of N'Dama cattle

Policy frameworks	Specific phrases relating to N'Dama cattle breed	Proposed strategies
1st 5-Year Development Plan (1975/76 - 1979/80)	Development of N'Dama cattle improvement programmes.	Use of artificial insemination involving crosses with exotic breeds.
2nd 5-Year Development Plan (1981/82 - 1985/86)	Facilitate the export of N'Dama cattle breeding stock to tsetse fly infested countries.	Enforcement of existing legislation and formulation of new ones if necessary.
Economic Recovery Programme - 1985	Handing over of the N'Dama nucleus in the DLS to the Livestock Marketing Board.	Commercialization of the livestock enterprise.
Programme for Sustained Development (1990 -1994)	Improvement of N'Dama production.	Advocacy for liberalized trade, private businesses and foreign investments.
Gambian Vision 2020	Increasing N'Dama cattle producers' off-take rate, access and coverage of livestock production services through private-public partnership.	Strengthening disease reporting, surveillance and monitoring systems; provision of quality feed, drinking points, and cattle tracks.
Poverty Reduction Strategy Paper I and II (2008-2011)	Improving animal health delivery and provision of AI services and feed concentrates for commercial dairy; intensification of fodder production.	Reorganize the livestock extension delivery system; mass vaccination campaigns; and training of technical advisers.
Agriculture and Natural Resources (ANR) policies (2009-2015)	Continuous exploitation of the highly productive and well adapted species of livestock.	National policies coordinated and harmonized with regional and international policies; Value chain approach to production, processing and marketing.
Gambia Agricultural Investment Plan (2011-2015)	Provision of specific incentives to e.g. N'Dama cattle farmers.	Improved rangeland infrastructure, feed availability and increased awareness.

A further elaboration of those policies with particular attentions on the aspects that relate to N'Dama cattle is shown in the table below. In Table 1, the sustainable management of N'Dama cattle for food production, income generation, employment opportunities and economic development have always been an integral part of different policy frameworks of The Gambia. Also, there are various policy options and strategies that are deployed to ensure optimal contributions of N'Dama cattle to increased food security and better livelihood of the citizens. Those policies and frameworks were also means to operationalize some international agreements and conventions which The Gambia had ratified or consented.

Discussion

With a specific reference to the N'Dama cattle, it is particularly interesting that this breed is still a choice of many resource-poor farmers. Nonetheless, it is important to save this unique breed from threats like indiscriminate crossbreeding and destruction of its natural habitats. The comparative performance of this breed especially in terms of lower quantity of daily milk production when compared to the Zebu and exotic breeds constitutes a factor that makes some farmers to carry out breed substitution and crossbreeding in certain production environments such as peri-urban areas. Drucker *et al.* (2001) reported that AnGR

erosion manifested in the form of animal breed replacement (substitution and crossbreeding) with a more suitable and highly productive breed is a significant factor in the global loss of farm animal biodiversity while limited access rights to grazing land and water resources was pointed out by Köhler-Rollefson (2005). Taking a look at the developing countries, it has been observed that the greatest risk of AnGR loss is found in these parts of the world for various reasons that could be summarized as socio-politically, economically, and agro-ecologically motivated (FAO, 2007a; Signorello and Pappalardo, 2003).

Concerning the ONBS for the N'Dama cattle, Fall *et al.* (2003) also reported that proper evaluation and phenotypic selection based on those farmers' desired traits would improve the production, productivity and competitiveness of animals in a cumulative manner while Bosso (2006) stated that the sustainability of this breeding scheme in The Gambia is feasible and could serve as a good model for both low and medium livestock production systems in the West African subregion. Furthermore, nucleus herds/flocks are the basis of breeding and genetic improvement programmes in most of the developing countries including those of Africa (Kosgey *et al.*, 2002). As an alternative approach to dissemination of elite bulls to the farmers, Olaniyan and Hiemstra (2012) had previously explored the option of artificial insemination (AI) but the authors however identified financial constraint, low success rates, complex logistical requirement, and farmers' inadequate knowledge of keeping animal breeding records as some of the challenges that may be encountered in using this reproductive technology in The Gambia. In addition, careful considerations should be given to the undesirable effect like inbreeding depression that may result from an inaccurate or unguided use of AI (van Arendonk, 2011). Another realistic option to explore would be the reorganization and proper orientation of the defunct GILMA towards real ownership of the N'Dama cattle breed. This should be done in addition to provision of financial, institutional, political and operational supports by the relevant government agency and other

stakeholders with interests in sustainable utilization and conservation of endemic ruminant species. Also, regular monitoring of the GILMA's activities by the relevant government institution with a view to offering them timely advice on statutory roles, financial management, independence and continuity would improve this organization's efficiency.

Interestingly, the ANR Policy and other related frameworks such as the National Trade Policy, Gambian Livestock Marketing Agency 2008 Act, and Poverty Reduction Strategy Paper I and II (2008-2011) have stimulated sustainable production and utilization of livestock species to a good extent. Meanwhile, it has been recognized that for a successful utilization and conservation of animal genetic resources (AnGR) which include the N'Dama cattle, there is a need for promotion of favourable public policies and legislations (FAO, 2007b; Hiemstra *et al.*, 2007). A comprehensive review of alternative policy instruments including the pros and cons for their implementation in the different live-stock-related domains was however proposed by Pica-Ciamarra *et al.* (2010). To address the crosscutting issues and conflicting interests of stakeholders, transparency and clearly defined objectives are also required in policy design and implementation stages. Hiemstra *et al.* (2007) also recommended an enabling environment for all relevant stakeholders as an option for strengthening policy and regulatory frameworks in sustainable AnGR utilization and conservation. For the policies that deal with how to sustain N'Dama cattle in The Gambia, all efforts should be made to embrace the principle of stakeholders' inclusiveness at every stage starting from the design to implementation.

There are other groups with stakes in ensuring N'Dama cattle production, utilization and conservation in The Gambia. These stakeholders according to Olaniyan and Hiemstra (2012) included N'Dama cattle owners, policymakers, scientists, veterinarians, agricultural officers and livestock technicians. However, there is a need to consider other actors along the livestock value chains in order to enhance an effective stakeholders' approach

to sustainable management. Such political, financial, intellectual, human and technical resources of these stakeholders are highly essential in ensuring inclusiveness in policy making. By properly harnessing the capabilities, roles and experiences of these stakeholders in combination with the farmers' willingness to continue the husbandry of N'Dama cattle, a participatory or collaborative approach to sustainable production and utilization of this cattle breed could be achieved. Moreover, the willingness of farmers to get informed on how to achieve improved production goals set for N'Dama cattle by the government should be capitalized upon as a stimulus for policy dialogue, design, awareness, and implementation. Effective and efficient means of communicating the objectives of relevant livestock policies through educational campaigns, orientation workshops, traditional communication media and importantly through local leaders or chiefs would be effective in this regard. Strengthening of available human capacity and institutional framework for transforming policies into projects is another relevant option. This same recommendation also put forward by FAO (2007b) and Hiemstra *et al.* (2006) apart from making the stakeholders to be better informed will also encourage collaborative efforts and adaptive management in terms of sustainable utilization and conservation of local breeds. Involvement of different category of stakeholders at every stage of policy design, wide dissemination of the resulting policy documents, creating awareness about its objectives through sensitization, publications and wide distribution would contribute to effectiveness of such policies. Meanwhile, FAO (2007a) warned that there may be conflicting interests of some stakeholders and cumbersome logistical operations may be involved. In this regard, policy targeting the sustainable production and conservation of N'Dama cattle in The Gambia has to be in line with the country's agricultural objectives and should capitalize on the existing institutions or particular ad-hoc committees as vital instruments in overall policy making and implementation. This process in addition to being cost effective will also help in filling the knowledge and information gaps of the

relevant stakeholders at the national level. Meanwhile, Olaniyan and Hiemstra (2012) reported that local protocols on forestry, wildlife and other environmental regulations of The Gambian government which were designed for natural resources management have indirectly stimulated cattle production and its conservation by the farmers and such model could be further explored.

Conclusions

Optimization of the open nucleus breeding scheme for N'Dama cattle as a means of providing genetically improved bulls for the farmers will enhance sustained genetic improvement, production and utilization of this endemic cattle breed. To ensure adequate supports for conservation of genetic traits in the N'Dama cattle, financial and technical capacity building of experts in the relevant institutions should be ensured. Also, transparency, inclusiveness and flexibility in terms of policy making and implementation as recommended by the relevant stakeholders should be considered. The N'Dama cattle breed can be adequately protected against any form of human activities that could lead to its endangerment in order to maximize its important traits that are needed for ensuring resource-poor farmers' access to animal protein, draught power and income generation.

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DESCRIPTIVE SURVEY OF PERSONAL HYGIENE AND KNOWLEDGE OF EXPOSURE FACTORS OF ZOOBOTIC DISEASES AMONG POULTRY WORKERS IN OGUN STATE, NIGERIA

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Abstract

A cross sectional survey was conducted using questionnaire as a tool from March to July, 2011 to determine the personal hygiene and safety status of some selected poultry workers in Ogun State, Nigeria. The questionnaire was also to determine the knowledge of poultry workers on various factors that can expose them to zoonotic diseases. A total of 143 questionnaires were administered. Sizeable number of poultry workers (74.8%), were not provided with personal protective equipment (PPE) during work. Majority of the poultry workers have adequate personal hygiene while use of antiseptics as part of hand washing practices is relatively low. Forty-three percent of poultry workers have some knowledge of occupational exposure factors and 27.3% have some knowledge of non-occupational exposure factors to zoonotic diseases. It was concluded that, there is a need for provision of adequate personal protective equipment, adequate decontamination and hygienic facilities for poultry workers as well as awareness creation and education of poultry workers on various exposure factors of zoonotic diseases.

Keywords: Personal hygiene, Knowledge of risk factors, Poultry workers, Zoonotic diseases

ETUDE DESCRIPTIVE DE L'HYGIÈNE PERSONNELLE ET DE LA CONNAISSANCE DES FACTEURS D'EXPOSITION AUX ZOOBOTES PARMI LES AVICULTEURS DE L'ÉTAT D'OGUN AU NIGERIA

Résumé

Une enquête transversale a été réalisée au moyen d'un questionnaire, de mars à juillet 2011, dans le but de déterminer la situation de l'hygiène personnelle et de la sécurité sanitaire de certains travailleurs avicoles sélectionnés dans l'État d'Ogun au Nigeria. Le questionnaire avait également pour objectif de déterminer les connaissances des travailleurs avicoles par rapport aux divers facteurs susceptibles de les exposer aux zoonoses. Au total, 143 questionnaires ont été administrés. Un nombre appréciable de travailleurs avicoles (74,8%) n'a pas été pourvu d'équipements de protection individuelle (EPI) au travail. L'on a noté que la majorité des travailleurs avicoles a une hygiène personnelle adéquate, mais que son utilisation d'antiseptiques dans le cadre de pratiques de lavage des mains est relativement faible. Quarante-trois pour cent des travailleurs avicoles ont une certaine connaissance des facteurs d'exposition professionnelle aux zoonoses, et 27,3% ont une certaine connaissance des facteurs d'exposition non professionnelle aux zoonoses. Il a été conclu qu'il est nécessaire de fournir des équipements de protection individuelle adéquats, de mettre en place les installations de décontamination et d'hygiène pour les travailleurs avicoles, de sensibiliser et de former ces derniers aux divers facteurs d'exposition aux zoonoses.

Mots-clés : hygiène personnelle ; connaissance des facteurs de risque ; aviculteurs ; zoonoses

Introduction

Biosecurity is defined as any practice or system that prevents the spread of infectious agents from infected to susceptible animals, or prevents the introduction of infected animals into a herd, region, or country in which the infection has not yet occurred (Radostits, 2001). This embodies all measures which can or should be taken to prevent viruses, bacteria, fungi, protozoa, parasites, disease carriers (rodents, insects, wild birds, people, equipment, etc) from entering and endangering the health status of a population. Biosecurity is an extremely important safety measure. Indeed, the lack of biosecurity on many farms is a key factor behind the persistence and transmission of some diseases between animals and to humans (WHO, 2005).

Human contact with poultry, both at the household and the industrial level, is a clear risk factor for exposure to avian commensals that can infect humans, including bacteria such as *Campylobacter* spp., *Salmonella* spp. and *Listeria monocytogenes*, as well as viruses such as those causing avian influenza (van Boven *et al.*, 2007). Epidemiological analyses of human infections originating from poultry have demonstrated that close interaction with domesticated live poultry is a risk factor for human infection with these disease agents (Babakir-Mina *et al.*, 2007). Growers, catchers and their families experience exposure to zoonotic pathogens from direct and indirect occupational contact with live birds and poultry manure. Among these populations, exposure to viral and bacterial pathogens may result from working in the confinement house itself, handling live chickens, cleaning the confinement house or transporting animal waste (van Boven *et al.*, 2007; Graham *et al.*, 2008).

In Nigeria, there is close interaction of poultry workers with poultry as their work descriptions involved routine packing of poultry dung, picking of laid eggs into the crates, feeding of birds, transportation of birds from one farm to another and from one poultry house to another. These conditions provide considerable risk of exposure to various zoonotic pathogens. Thus, the level of knowledge of risk factors

of exposure and biosecurity status of these workers is unknown and needs to be assessed.

Therefore, this study has been conducted to determine the biosecurity status of the poultry workers and to obtain an in-depth understanding of knowledge of exposure to risk factors of these workers to zoonotic diseases in some selected poultry farms in Ogun State.

Materials and Methods

Study location

The study was carried out in some selected poultry farms within the four zones in Ogun State. Ogun State consists of 20 Local Government areas divided into four zones namely Egba, Ijebu, Yewa and Remo. It lies between latitude 6.2°N and 7.8°N and longitude 3.0°E and 5.0°E at an elevation of 169 feet with an area of 16,762 square kilometers and 4,054,272 populations (Adebowale *et al.*, 2010).

Study design

A cross-sectional survey was conducted from March to July 2011. The survey was carried out using a semi-structured questionnaire. A total of 143 respondents were interviewed, the workers were interviewed confidentially in their workplace regarding socio-demographic characteristics, personal hygiene and safety status, general knowledge of occupational and non – occupational exposure factors of zoonotic diseases. All interviews were undertaken on spot in the farms.

Data Analysis

Data collation and management was done on Microsoft Excel. Responses to questionnaire were presented in simple frequency.

Results

The demographics data of the respondents indicated that poultry work is common among youths (20 - 30 years) and the majorities were male (67.8%). About half of the respondents had attended up to

secondary school and less than 5% had no formal education. Most of the respondents (73.4%) were of religion of Christianity while one-quarter of Muslim religion. Relatively most workers have less than one year experience on poultry work (Table-1).

Majority of the poultry workers (74.8 %) were not provided with personal protective equipment (PPE) but use their personal work clothes for each day work. Majority (79.4%) of the poultry workers come in contact with poultry faeces while 45.5% do have contact everyday (Table -2).

Many workers (97.9%) wash and reuses the protective coverings and work clothes. While most (93.7%) of the workers wash their hands immediately after contact with birds or

faeces, 69.2% wash their hands always. Hands are mostly washed with water and detergents, many of the poultry workers (88.1%) take their bath after each work period. While almost 10% of the poultry workers eat during work period, few (11.9%) of them do so within the poultry house. Majority (95.1%) of the workers wash their hands before eating, use of cutleries is however common (90.2%) among the poultry workers.

From the survey, close to half of the poultry workers were aware of some occupational exposure factors of zoonotic diseases (43.4%); possible ways of exposure stated are shown in Table 3. While 27.3% of poultry workers were aware of some non-occupational exposure factors (Table 3).

Table I: Socio-demographic status of the respondents

	Socio-demographic characteristics	Frequency	Percentage (95%CI)
Age in years	(A)		
	< 20	18	12.6 (7.0-19.0)
	20 – 30	66	46.2 (37.7-54.7)
	31 – 40	46	32.2 (24.6-40.5)
	41 – 50	13	9.0 (4.9-15.0)
Sex	(B)		
	Male	97	67.8 (59.5-75.4)
	Female	46	32.2 (24.6-40.5)
Religion	(C)		
	Islam	37	25.9 (18.9-33.9)
	Christianity	105	73.4 (65.4-80.5)
	Traditional	1	0.7 (0.0-4.0)
Educational level	(D)		
	Non-formal education	6	4.2 (1.6-8.9)
	Primary school cert.	15	10.5 (6.0-16.7)
	Secondary school cert.	77	53.8 (45.3-62.2)
	Tertiary education	45	31.5 (24.0-39.8)
Number of years on the job	(E)		
	< 1	55	38.4 (30.5-47.0)
	1 – 2	34	23.8 (17.1-31.6)
	3 – 5	25	17.5 (11.7-24.7)
	5 – 10	17	11.9 (7.1-18.4)
	>10 years	12	8.4 (4.4-14.2)

Table 2: Personal hygiene and Safety status of poultry workers in the selected poultry farms in Ogun state (n=143).

Items	Response	Frequency	Percentage(95%CI)
(A) Protective coverings	Yes	36	25.2 (18.2-33.0)
	No	107	74.8 (66.9-81.7)
Yes	Foot wear	21	14.7 (9.3-21.6)
	Nose mask	20	14.0 (8.7-20.8)
	Hand glove	22	15.4 (9.9-22.4)
	Farm coat	29	20.3 (14.0-27.8)
	personal clothes	107	74.8 (66.9-81.7)
Washing of covering/work clothes	Yes	140	97.9 (94.0,99.5)
	No	3	2.1 (0.4-6.0)
(B) Contact with poultry faeces	Yes	114	79.7 (72.2-86.0)
	No	29	20.3 (14.0-27.8)
Frequency of contact	Everyday	65	45.5 (37.1-54.0)
	Occasionally	49	34.3(26.5- 42.7)
(C) Washing of hands after contact	Yes	134	93.7(88.4- 97.1)
	No	9	6.3 (2.9- 11.6)
Frequency of hand washing	Always	99	69.2 (60.9-76.7)
	Occasionally	35	24.5 (17.7- 32.4)
	Never done	9	6.3 (2.9- 11.6)
Method of hand-washing	Ordinary water	12	8.4 (4.4- 14.2)
	Detergent and water	61	42.7 (34.4-51.2)
	Detergent, water and antiseptics	61	42.7(34.4-51.2)
(D) Bath taking after work period	Yes	126	88.1 (81.2- 92.9)
	No	17	11.9 (7.1- 18.4)
(E) Eating habit during work period	Yes	114	79.7 (72.2-85.9)
	No	17	18.9 (7.1-18.4)
	No response	12	8.4 (4.4-14.2)
Place of eating	Within poultry house	17	11.9 (7.1-18.4)
	Special room	97	67.8 (59.5-75.4)
How do you eat	Bare hands	14	9.8 (5.5-15.9)
	Cutleries	129	90.2 (84.1-94.5)
Washing of hands before eating	Yes	136	95.1 (90.2-98.0)
	No	7	4.9 (1.9-9.8)
Frequency of hand washing	All the times	111	77.6 (69.9-84.2)
	Sometimes	25	17.5 (11.6-24.7)

Table 3: General knowledge of exposure factors of zoonotic diseases among the poultry workers

Items	Response	Frequency	Percentage(95%CI)
(A) knowledge of occupational exposure	Yes	62	43.4(35.0-52.0)
	No	81	56.6 (48.1-64.9)
Yes	Unwashed hands	5	3.5 (1.2-8.0)
	Lack of hygiene	25	17.5(11.6-24.7)
	Contact with poultry faeces	4	2.8 (0.7-7.9)
	Inhalation of poultry dust	5	3.5 (1.2-8.0)
	Contact with infected birds	8	5.6 (2.4-10.7)
	(B) Knowledge of non-occupational exposure	Yes	39
Yes	No	104	72.7 (64.7-79.8)
	Unhygienic environment	12	8.4 (4.4-14.2)
	Improper cooked food	3	2.1 (0.4-6.0)
	Lack of hygiene	33	23.1(16.4-30.9)
	Contaminated water	3	2.1(0.4-6.0)

Discussion

The demographics data of the respondents indicated that they were generally youths (20-30 years) which denote age range of vigour and strength and the majority were male which is consistent with the findings of Fawole (2006) and Fatiregun (2008) who reported an average age of respondents was 36 years and 30 years respectively among poultry workers in Oyo State.

Biosecurity is an extremely important safety measure whether at personal or poultry farm level, as human contact with poultry, is a clear risk factor for exposure to zoonotic disease that can infect poultry workers (van den Bogaard *et al.*, 2002). Our work disclosed that, many poultry workers are not provided with personal protective equipment when entering the poultry house but have special work clothes which they wash from time to time, despite the fact that majority of poultry workers come in contact with poultry faeces; which can serve as reservoirs of major food-borne zoonotic diseases. This might indicate the possibility of risk of exposure of workers to zoonotic agents and other work related hazards. This finding have been reported elsewhere in Nigeria (Fatiregun, 2008; Fasure

et al., 2012,) and in America (Graham *et al.*, 2008) which suggest that this finding is not limited only to developing countries. There is an adequate to excellent practice of personal hygiene among poultry workers as observed in this study as greater percentage practice washing of work clothes, washing of hands after contact with poultry, regular bathing taking and washing of hands before eating any type of food. This may suggest the high level of basic hygiene awareness among workers which was applied in their works however; there is inadequate provision of decontamination facilities such as antiseptics, as less than 50% of poultry workers have access to antiseptics. In agreement with our finding, Graham *et al.* (2008) reported that, there is typically little or no cleaning and hygiene facilities provided to poultry workers in United State of America and Fasure *et al.* 2012 observed that use of decontamination facilities were not adhere to by poultry workers. Also, studies elsewhere had demonstrated that conditions such as improper hand washing and indiscriminate eating in animal facilities, are some of the factors responsible for the spread and circulation of zoonotic pathogens in poultry farms (Snoeyenbos, 1991; Wright *et al.*, 2005), findings that were common among some poultry workers of this study as 11.9%

eat within poultry facilities during working periods.

Despite majority of the poultry workers have secondary and post-secondary education; there is average to poor knowledge of occupational and non-occupational risk factors of exposure to zoonotic diseases. This finding has also been reported by Swai *et al.* (2010) among livestock keepers in Tanzania. This implies the possibilities of poultry workers being at risk of work related hazards especially infectious agents, because knowledge about risk factors of exposure is very much essential to reduce the chances of exposure and transmission of zoonotic diseases as knowledge is power however, lack of hygiene was the commonest risk factor stated by most poultry workers for both occupational and non-occupational factors.

Finally, this survey revealed that, there is need for provision of adequate personal protective equipment, adequate decontamination and hygienic facilities for poultry workers as well as awareness creation and education of poultry workers on various risk factors of exposure to zoonotic diseases.

Acknowledgement

We appreciate all the poultry managers and poultry workers interviewed for their cooperation and time.

Impact

This study has provided information on hygiene status of poultry workers with this region as well as their knowledge of exposure to zoonotic diseases. Also, this research has shown the poor biosafety status as well as inadequate knowledge of factors that could exposed these workers to zoonotic diseases.

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THE INCIDENCE AND PATTERN OF PNEUMONIA IN PIGS SLAUGHTERED AT THE KUMASI ABATTOIR, GHANA

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Abstract

Pneumonia has been identified as a major disease condition limiting the swine production in West Africa; however the data on its implications to swine health has not been fully elucidated. The pattern and prevalence of pneumonia in slaughtered pigs was determined in respect to the age, sex and breed of pigs slaughtered between a periods of four months (November 2013-February 2014). Samples of the affected lungs were also taken for histopathological analysis using standard techniques. Of 2,462 pigs were slaughtered, 665 pigs were purposefully examined and 33 (5%) were found to have pneumonia. The consolidation was more to the left lung than right while the histological classification showed more of the bronchopneumonia with more peribronchiolar reactions buttressing the cranial lobe consolidation observed in all the breeds. The intact males were more affected than castrates and female while more pneumonia cases were observed in Landrace than Large White and the indigenous Ashanti Black pigs. The younger and aged animals were also more affected. Though the prevalence was more to the late raining season and early dry season, the direct financial loss was 2.5 Million cedis for every one million pig slaughtered. With the indirect financial loss due to weight loss, anorexia and cost of treatment, this loss could be enormous. Hence, there is need for an extensive investigation to identify the causal agents of pig pneumonia in Ghana using isolation and immunohistochemistry techniques in order to initiate the appropriate control strategy to curtail pig pneumonia.

L'INCIDENCE ET LA TENDANCE DE LA PNEUMONIE CHEZ LES PORCS ABATTUS À L'ABATTOIR DE KUMASI AU GHANA

Résumé

La pneumonie a été identifiée comme une affection majeure limitant la production porcine en Afrique de l'Ouest ; cependant, les données sur ses implications pour la santé porcine n'ont pas été complètement élucidées. La tendance et la prévalence de la pneumonie chez les porcs abattus ont été déterminées par rapport à l'âge, au sexe et à la race, sur une période de quatre mois (Novembre 2013-Février 2014). De plus, des échantillons de poumons affectés ont été prélevés pour une analyse histopathologique utilisant des techniques standard. De l'ensemble des 2.462 porcs abattus, 665 ont été examinés à cette fin ; et l'on a découvert que 33 (5%) avaient une pneumonie. Les opacités étaient plus notées pour le poumon gauche que le poumon droit, tandis que la classification histologique a montré un nombre plus élevé de cas de broncho-pneumonie avec des réactions pérbronchiolaires étayant les opacités du lobe crânienne observée dans toutes les races. Les mâles entiers étaient plus affectés que les castrats et les femelles ; et un nombre plus élevé de cas de pneumonie ont été observés chez les Landrace par rapport aux Large White et aux porcs Ashanti noirs indigènes. Les animaux plus jeunes et plus âgés ont été également plus touchés. Même si la prévalence était plus élevée à la fin de la saison des pluies et au début de la saison sèche, la perte financière directe enregistrée était de 2,5 millions de cedis pour chaque million de porcs abattus. Cependant, compte tenu de la perte financière indirecte occasionnée par la perte de poids, l'anorexie et le coût du traitement, cette perte pourrait être énorme. Par conséquent, il est nécessaire de mener une enquête approfondie pour identifier les agents étiologiques de la pneumonie porcine au Ghana en utilisant des techniques d'isolement et d'immunohistochimie afin de lancer la stratégie de contrôle appropriée pour endiguer la pneumonie porcine.

Introduction

Agriculture in Ghana consist of crop and livestock production, however livestock production has for the past years taken center stage in the agricultural sector of Ghana. The livestock subsector of Ghana which forms about 6.1% of GDP has a livestock and poultry population totaling more than 36 million which consists of 1454000 cattle, 3759000 sheep, 4855000 goats, 536000 pigs and 47752000 poultry and has domestic meat production of 112067 metric tons (MT), consisting of 19990MT Beef, 16914MT Mutton, 18935MT Chevon, 18026 Pork and 38202 MT poultry. This short fall in production is often augmented by meat import of 12483MT of beef, 2699.5MT of buffalo meat, 4902.6MT of chevon, 3038MT of pork, 70900.2MT of duck (VSD, MoFA, 2010).

The increasing population rate of Ghana is currently pegged at 3% and the human population is nearly 25 million. Unfortunately, the rate of growth of the animal population has not kept pace with that of humans. Ajayi (1975) stated that the increase in the size of human population is always at the expense of wildlife and livestock production. the obvious short fall in meat production which could be filled with high meat yielding animals like pigs with improvement in the management systems to prevent economic important diseases.

Meat which is considered as a choice food of great nutritional value is an excellent good biological protein and a main source of many nutrients, especially B vitamins, iron and zinc. (Ahmed *et al*, 2012). With the deficit in the daily protein intake, pork consumption gradually gained roots in most Ghanaian households as one of the main source of protein.

Despite all these advantages, the swine industry is plagued with diseases, some of which are of public health concerns includes African swine fever, foot and mouth disease, hog cholera, Swine vesicular disease and transmissible gastroenteritis, others include conditions such as pneumonia, pleuritis, porcine chronic pericarditis and tuberculosis. The importance of some of these diseases that could result into significant economic losses resulting from condemnation of edible organs

and carcasses has not being elucidated in Ghana, hence the need to ascertain the prevalence and pattern of pneumonic consolidations with swine pneumonia.

Materials and Methods

Study area

One of the principal functions of a modern abattoir is to provide efficient facilities for the slaughter of livestock, meat inspection procedures and refrigeration to ensure the production of wholesome meat. Kumasi abattoir is one of abattoirs in developing countries to which animals are supplied from farms within and outside a country. Animals such as pigs destined for slaughter at the Kumasi abattoir are transported from other districts of the Ashanti region such as Ejisu and also from other regions in Ghana, including the Northern region. The Kumasi abattoir is a transit point where all the animals transported from the Northern region which is the principal provider of Ghana's livestock as well as from all the neighbouring countries such as Burkina Faso, Mali, and Niger are rested before taken to the Accra, the capital of Ghana and other regions. Kumasi is also plays a major role in the food chain of Ghana because it is densely populated with the working class who has the means to afford animal protein. The northern region of Ghana has a larger percentage of its population being Muslims and for that reason, all the pigs produced are sent down to Kumasi where it has a ready market due to the lower percentage of Muslims. 50-60% of pigs in Ghana are concentrated in the northern sector (Ashanti and Brong-Ahafo regions inclusive) of the country and over 90% of this lot is made up of Ashanti Black Pig (Barnes, 1994, Ahunu *et al*, 1995). It is against this backdrop, that the Kumasi abattoir was chosen for this study.

Kumasi abattoir and slaughtered animals

The study area has been earlier described by some workers (Frimpong *et al*, 2012). Briefly, the Kumasi Abattoir is located at 6°39'36.6"N Latitude and 1°36'15.4"W Longitude, in Kumasi, the second capital city of Ghana. The Kumasi Abattoir Company Limited

was established in 1997 with grants from the Government of Ghana and the Canadian International Development Agency (CIDA) and commenced its operations in 1998 but is it currently managed by a private firm.

There is a holding pen for animals which are destined for slaughter within the next few days and these pens are used by the pig farmers and dealers to house their animals which are brought in to be sold. Although, the Kumasi abattoir slaughters animals like cattle, pigs, sheep and goats for processing and packaging for the targeted Ghanaian markets, the slaughter animals considered in this study were only pigs. The original daily slaughtering capacity of the Abattoir was 200 cattle, 100 pigs, and 250 sheep and goats. In addition to this, the production facility of the abattoir has been re- designed to enable the Company slaughter up to 400 cattle daily. This was achieved by converting one of the slaughter lines meant for the slaughter of sheep and goats into another cattle line and developing a singeing platform.

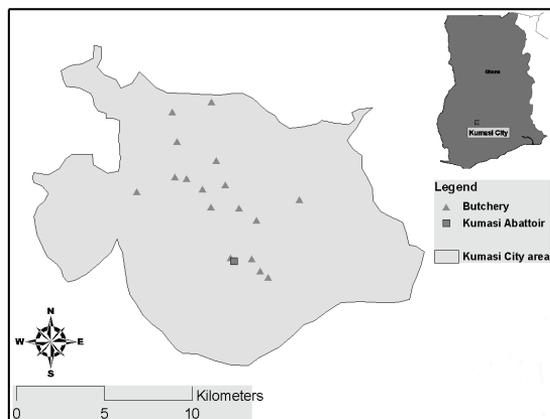


Figure 1: A map showing the Kumasi abattoir

Study animals.

The study was started in November, 2013 to March, 2014. During this period, five hundred and sixty (665) slaughtered pigs of Landrace, Ashanti black pigs and Large White breed were examined for pneumonia. Out of this number of animals examined, 48% were Large White whilst 40% were Landrace and the remaining 12% were the Ashanti black pigs.

Sampling technique.

A purposeful sampling technique was employed in this study where all the lungs of the pigs slaughtered during the period of the study were thoroughly examined for consolidation being the hallmark of pneumonia. Important study variables like age, sex, breed and location of lesions such as lobar and type of lung distribution. The affected portions were shaded on a designed card.

Post-mortem examination

The extent of pneumonia was determined by visual observation, palpation, making incisions where necessary for the presence of pus and other abnormalities. Lesion is manually plotted onto a lung diagram and then estimated as a percentage of each lobe. Lung consolidation as a percentage of the total lung volume was estimated as earlier described by Emikpe B.O and Akpavie (2010) for goat. The percentage of lung mass constituted by each lobe of a normal lung is as follows: left anterior cranial lobe 5%, left anterior cranial 6%, accessory lobe 4%, right cranial lobe 6%, right posterior cranial lobe 5%, right middle lobe 7% and right caudal lobe 35%, left caudal 32%. Using these values and the percentage pneumonic tissue in each lobe, the total percentage of pneumonic tissue was calculated for each animal and recorded as the lung lesion score. A fresh portion of the pneumonic lungs were taken for bacterial isolation while the other was preserved in 10% formalin for histological examination. The economic loss in terms of meat yield was estimated as described by Borji *et al.* 2011.

Statistical analysis

Data analyses were carried out using simple descriptive statistical methods.

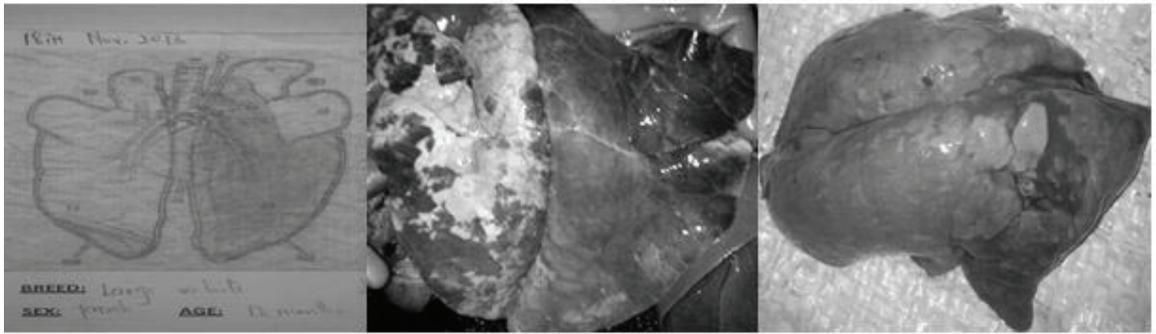


Figure 2: A replica of the lung designed to collect data, and some pneumonic lungs of pigs slaughtered at the Kumasi Abattoir.

Results

Prevalence of pneumonia

From the data gathered from November 2013 to February 2014, 2,462 pigs were slaughtered at the Kumasi abattoir and 665 slaughtered pigs were purposefully examined for pneumonia, out of which 33 which represents 5% were found to be pneumonic.

Sex influence on the prevalence of pneumonia.

The number of males both castrates and intact examined was 30% of the total examined as against 70% of female as shown in figure 3

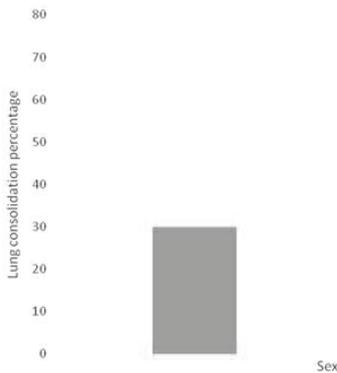


Figure 3: A graph showing the sex influence on the prevalence of pneumonia in slaughtered pigs

Breed influence on the prevalence of pneumonia in slaughtered pigs.

It was observed that the landrace had the higher percentage of the pneumonic lesions compared to the Large White and the indigenous Ashanti Black pigs as shown in figure 4

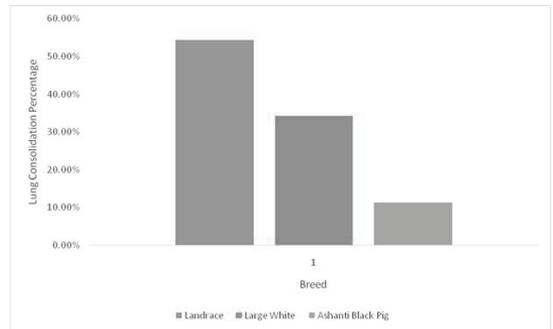


Figure 4: A graph showing the breed influence on the prevalence of pneumonia.

Age influence on the prevalence of pneumonia in slaughtered pigs.

It was observed that age has an influence on the pattern of pneumonia. The younger animals which fall within the age range of 6-12 months recorded higher incidence of pneumonia. High lung consolidations were recorded in pigs that fell within the age ranges of 31-36 months as compared to those that fall within the age ranges of 13-18 months, 19-24 months and 25-30 months as shown in figure 5.

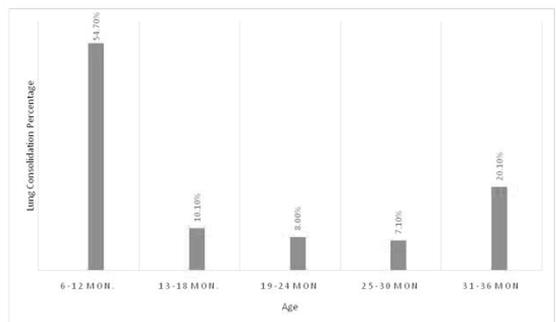


Figure 5: A graph showing the age influence on the prevalence of pneumonia in slaughtered pigs

Lung consolidation pattern in slaughtered pigs.

The left lobe of the lung had the highest percentage of the lesions as shown in figure 6.

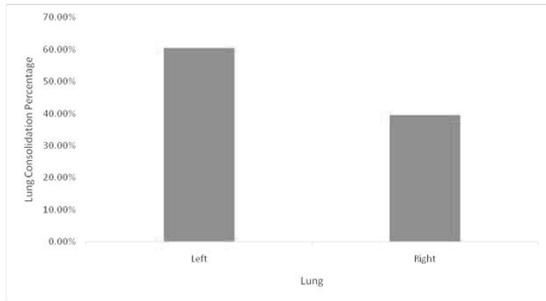


Figure 6: A bar chart showing the lung pattern of pneumonia in slaughtered pigs.

Lobar pattern of pneumonia in the three breeds of slaughtered pigs.

There was more cranial lobes consolidation across all the breeds which were indicative of bronchopneumonia as shown in figure 7.

Seasonal influence on the prevalence of pneumonia.

Out of the 33 cases of pneumonia recorded in this study, 12 of the cases which

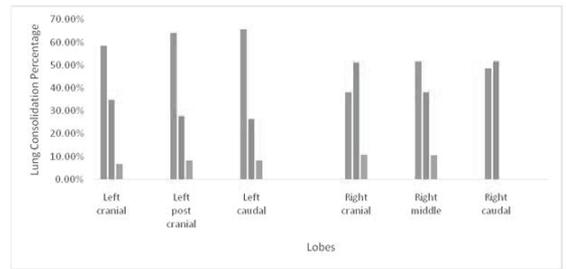


Figure 7: A bar chart showing the lobar pattern of pneumonia in slaughtered pigs

represent 36.6% were recorded in the rainy season i.e. in the month of November and 15 cases were recorded in the month of February, which also represents 45.5%. 2 cases, representing 6.1% were recorded in December while 4 cases, representing 11.8% were recorded in the month of January. Histopathology

The pattern of pneumonia was characterised by peribronchiolar lymphoplasmatic infiltrations which suggest mycoplasma pneumonia with bronchoectasis as shown in figure 8.

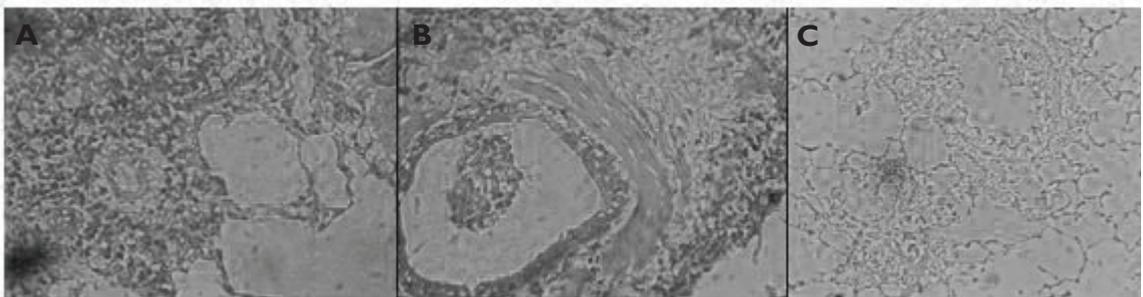


Figure 8: showing histological changes associated with bronchopneumonia, A: with intra alveolar and perivascular neutrophilic infiltration, B: with intrabronchiolar purulent exudates and C: peribronchiolar inflammatory cellular infiltration.

Discussion

This study described the prevalence and pattern of pneumonia in slaughtered pigs in Kumasi abattoir of Ghana. 33 of the 665 slaughtered pigs had pneumonia, which represents 5% prevalence. Although the percentage prevalence of pneumonia in pigs varies from country to country, about 38-100% of pigs has been reported to have pneumonic

lesions in Finland where there is widespread use of vaccines against enzootic pneumonia (EP), the causal agent *Mycoplasma hyopneumoniae* (Guerrero, 1990).

From this study, it was observed that the intact males are more likely to be affected when compared to their castrates and female counterparts, this observation may be related with the active, often inquisitive and sexually active nature of the intact male when compared

with castrate and females. The influence of the breed on the occurrence of pneumonia in this study clearly showed the hard and tolerant nature of the Ashanti black pig to most diseases and the ability to survive under poor management and extremes of environmental conditions (Ahunu *et al.*, 1995; Darko and Buadu, 1998). The study also showed that Land race is more susceptible to pneumonia than Large Whites and the Ashanti Black Pigs. This observation may need to be further clarified.

On age influence, the younger animals which fall within the age range of 6-12 months recorded higher incidence of pneumonia. This observation may be related to the state of development of the immune system at this age and the preponderance of pneumonia within these age groups may have to do with the fact that once a herd is infected with *M.hypopneumoniae*, there are always pigs of susceptible age which continue to harbour the infection and that the disease agent cannot be eliminated by medication, although vaccination tend to reduce pneumonic lesions (Dohoo and Montgomery, 1996; Zhang *et al.* 1994; Djordjevic *et al.* 1997). Another very important peculiarity is the poor sanitation program in most pig farms around Kumasi and the fact that the restocking program is often not planned.

In terms of pattern of pneumonic consolidation, the left lobe of the lung had the highest percentage of the lesions. This pattern is often associated with mycoplasmal pneumonia which was further buttressed by the peribronchiolar nature of the reaction observed at histology. There was also more consolidation in the cranial lobes across all the breeds which were indicative of a bronchopneumonia as observed by other workers (Djordjevic *et al.*, 1997).

In terms of seasonal variation, the investigation revealed that the late raining season and early dry season recorded more pneumonia which corresponded with that observed in our studies in goats (Asare *et al.*, In preparation). The histological pattern was that of bronchopneumonia with peribronchiolar lymphoplasmatic infiltrations and bronchoectasis. This further shows the need for further investigation on the isolation

and immunohistochemistry to demonstrate the causal agents of pig pneumonia in Ghana.

Conclusion

The study revealed that there is sex, breed, age and seasonality influence on the prevalence of pneumonia in slaughtered pigs. Its worthy to note that more sows were slaughtered at the Kumasi abattoir than boars which obviously showed the need for urgent improvement on swine production systems in Ghana with drastic steps taken to minimize the slaughtering of the sows which are often the prime breeders. It is therefore recommended that control measures be put in place to reduce the prevalence of swine pneumonia through the use of appropriate vaccinations and antibiotic for prophylactic treatment in piglets and weaners.

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PERFORMANCE AND BONE MORPHOMETRY OF TWO BREEDS OF FINISHING BROILER CHICKENS REARED ON DIFFERENT HOUSING SYSTEMS

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Abstract

Adequate consideration of management systems is required to enhance the productivity of broiler chickens. This study was carried out to determine the effect of two housing systems on growth performance, carcass traits and bone morphometry of broiler chickens. A total of 180 unsexed one day-old Arbor Acre and Marshall breeds of broiler chickens were used for this study. The birds were brooded for 3 weeks and thereafter acclimatized for one week in their respective housing systems (deep litter and outdoor run). Each of the housing systems was allotted 90 chicks per breed with three replicates of 15 birds each. The birds were fed same quality and quantity of feed ad libitum and given water throughout the period of the experiment which lasted for four weeks. Data were collected weekly on weight gain and feed intake while feed conversion ratio was calculated. At the end of the study, 2 birds of representative weight in each replicate were selected for carcass analysis and bone strength determination. The experiment was arranged in a 2 x 2 factorial layout in a completely randomized design. The results of growth performance showed significantly ($p < 0.05$) higher final weight (2,012.06g/b), weight gain (48.45g/b/d) and feed intake (151.74g/b/d) in Arbor Acre breed compared with the final weight (1,890.15g/b), weight gain (45.82g/b/d) and feed intake (144.60g/b/d) obtained in Marshall breed. Significantly ($p < 0.05$) higher weight gain (49.70g/b/d) and better feed conversion ratio (2.92) was observed in broiler chickens reared on deep litter housing system while those reared on outdoor run had 49.11g/b/d and 3.01 for weight gain and feed conversion ratio, respectively. Birds managed on outdoor run consumed more feed compared to those kept on deep litter. Broiler chickens reared on deep litter were superior in retail cut (breast) and abdominal fat compared to those kept on outdoor run. Irrespective of breeds, birds reared on outdoor run had greater ($p < 0.05$) bone strength (14.61N/mm²) compared to those on deep litter (10.68N/mm²). The study concluded that, Arbor Acre breed of broiler chickens could be reared on deep litter housing system for better performance. However, outdoor run could be adopted to enhance better bone strength in both breeds of broiler chickens.

Keywords: Broiler chickens, breeds, performance, carcass traits, morphometry, housing systems

PERFORMANCE ET MORPHOMÉTRIE OSSEUSE DE DEUX RACES DE POULETS DE CHAIR D'ENGRASSEMENT ÉLEVÉS DANS DIFFÉRENTS SYSTÈMES DE LOGEMENT

Résumé

La prise en compte adéquate des systèmes d'élevage est nécessaire pour améliorer la productivité des poulets de chair. La présente étude a été réalisée pour déterminer l'effet de deux systèmes de logement sur la performance de croissance, les caractéristiques de carcasse et la morphométrie osseuse des poulets de chair. Au total, 180 poussins de chair d'un jour, des deux sexes, des races Acre Arbor et Marshall, ont été utilisés pour cette étude. Les oiseaux ont été couvés pendant 3 semaines et ensuite acclimatés pendant une semaine à leurs différents systèmes de logement (litière profonde et libre parcours). L'étude a assigné à chaque système de logement 90 poussins par race, avec trois répétitions de 15 oiseaux chacune. Les oiseaux ont reçu la même qualité et quantité d'aliments ad libitum et de l'eau tout au long de la période d'étude qui a duré quatre semaines. Chaque semaine, les données ont été recueillies sur le gain pondéral et

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la prise alimentaire, et le taux de conversion alimentaire a été calculé. A la fin de l'étude, 2 oiseaux de poids représentatif de chaque répétition ont été sélectionnés pour l'analyse de carcasse et la détermination de la solidité des os. L'étude a été réalisée selon une configuration factorielle 2 x 2 dans un schéma complètement aléatoire. Les résultats de la performance de croissance ont montré une augmentation significative ($p < 0,05$) du poids final (2,012,06g b / l), du gain pondéral (48,45g/b/d) et de la prise alimentaire (151,74g/b/j) chez les poussins Arbor Acre par rapport au poids final (1,890,15g / b), gain pondéral (45,82g/b/d) et prise alimentaire (144,60g/b/d) notés pour les poussins Marshall. Un gain pondéral significativement ($p < 0,05$) plus élevé (49,70g/b/d) et un meilleur ratio de conversion alimentaire (2,92) ont été observés chez les poussins élevés dans le système de logement à litière profonde tandis que ceux élevés en libre parcours ont eu un gain pondéral de 49,11g/b/d et un ratio de conversion alimentaire de 3,01. Les oiseaux élevés en libre parcours ont consommé plus d'aliments par rapport à ceux élevés sur litière profonde. Les poulets de chair élevés sur litière profonde avaient des coupes de viande au détail (poitrine) et des graisses abdominales plus importantes par rapport à celles des poulets élevés en libre parcours. Indépendamment des races, les oiseaux élevés en libre parcours avaient une plus grande ($p < 0,05$) solidité osseuse (14,61N/mm²) par rapport à ceux élevés sur litière profonde (10,68N/mm²). L'étude a conclu que les poulets de chair de race Arbor Acre pourraient être élevés en système de logement à litière profonde pour de meilleures performances. Cependant, l'élevage en libre parcours peut être adopté pour améliorer la solidité des os chez les deux races de poulets de chair.

Mots-clés : poulets de chair ; races ; performance ; caractéristiques de carcasse ; morphométrie ; systèmes de logement

Introduction

Broiler chickens production is no doubt one of the fastest growing segments of the poultry industry. It is perhaps the most significant source of quality protein and income as compared with other livestock (Linje, 2005). This is brought about by the increasing and persistent demand for poultry products like meat, eggs and even litter used as manure for crop production. However, one of the major production constraints bedeviling the poultry industry is in the area of housing.

Housing systems for poultry range from small, pasture-based flock to large, commercial-scale operations that intensively confine tens of thousands of birds indoor. Regardless of how birds are managed in the intensive production system, it is a known fact that birds might compromise important comfort movements. It is clear that this deprivation poses a serious problem on meat-type poultry and cage birds compared to the alternative housing system, thereby causing high incidence of bone problems which include bone weakness, deformity, breakage and infection and osteoporosis-related mortalities. Birds with deformed limbs have walking problems and their sitting on the bedding also causes malformations of thoracic muscles, affecting the

quality of meat and the profitability of poultry production (Rath *et al.*, 2000). In addition to this situation, broiler chickens with weak legs have worse performance because they have reduced feed intake (Brenes *et al.*, 2003). This often leads to problems of bone breakage during handling and transportation and during processing at slaughter house (Knowles and Wilkins, 1998). Therefore, in order to arrest this trend, emphasis directed towards research on different housing systems and technologies for chickens with adoptable recommendations for resource-poor poultry farmers are attracting increasing interest (Sogunle *et al.*, 2013) with a view to eradicate the problem of bone strength weakness and reduce economic losses of the present-day poultry breeding and processing industries. It is against this background that this study was designed to assess the effect of different housing system on the performance and bone strength of two breeds of broiler chickens.

Materials and Methods

Experimental site

The experiment was carried out at the Directorate of University Farms, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. The location falls within

the rainforest zone of South-Western Nigeria at longitude 70 10' 37" N, latitude 30 26' 58" E and altitude 173m above sea level. The climate is humid with a mean annual rainfall of 1,037mm. The mean annual temperature and humidity are 34.7°C and 82%, respectively (Federal University of Agriculture, Abeokuta, Meteorological Station).

Experimental animals and management

A total of 180 unsexed one day-old Arbor Acre and Marshall breeds of broiler chicks (90 birds per breed) were procured from a reputable hatchery. Prior to the arrival of the chicks, the brooding house was cleaned, washed, disinfected and dried before use. The chicks were floor brooded for 3 weeks, and thereafter acclimatized for one week in their respective housing systems viz. deep litter and outdoor run. The birds after balancing for body weights were distributed into 4 experimental groups of 45 birds each per treatment and randomly assigned to the housing systems. They were further divided into 3 replicates of 15 birds each. The trial lasted for 4 weeks (finishing phase), during which same quality and quantity of feed and clean, cool drinkable water were offered ad libitum. All medications and vaccination schedules were strictly adhered to.

The deep litter housing system was constructed as an open-sided house and fully enclosed to protect birds from predators. The floor was covered with wood shavings and had a dimension of 3m x 3m. In the outdoor run, a mini shelter was provided and birds were managed in an area (5m x 5m) that enabled them have access to adequate space and sunlight during the day.

Data collection

Determination of growth performance

- i. Body weight gain- The initial body weights of each group of birds were taken; thereafter, subsequent body weight was recorded on weekly basis.

$$\text{Body weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

- ii. Feed intake- A known quantity of feed was fed to birds while the leftover was weighed

to determine the daily feed intake and consequently weekly feed intake.

$$\text{Feed intake/bird} = \{\text{Feed supplied (g)} - \text{Feed leftover (g)}\} / \text{Total number of birds}$$

- iii. Feed conversion ratio (FCR) - The feed conversion ratio of each group of birds was determined by calculating the ratio of feed intake to weight gain.

$$\text{FCR} = \text{Total feed consumed (g)} / \text{Total body weight gain (g)}$$

- iv. Percentage mortality = $\{\text{Total number of dead birds} / \text{Total number of stocked birds}\} * 100$

General observation of birds was made on daily basis as a preventive measure to check for clinical signs of diseases or its predisposing factors as well as vice habits among the flock.

Carcass analysis

At the end of the trial, 2 birds weighing close to average of birds from each replicate were selected for carcass analysis and fasted for 12 hours. The birds were tagged and sacrificed humanely by severing the carotid arteries with subsequent exsanguinations. They were scalded, de-feathered, eviscerated and dissected to obtain carcass weights. Dressed carcasses, cut-up parts and visceral organs were weighed using electronic weighing scale. Carcass parameters taken were the plucked weight, dressed weight, dressing percentage, cut-up parts and organ weights. All cut-up parts and organ weights were then expressed as percentages of the live weight.

Bone

At the end of the experiment, shanks from fresh carcasses of one bird per replicate were excised and de-fleshed. The bones were individually sealed in sample bags to minimize moisture loss. Thereafter, the sample bags were placed in a plastic container and stored in a cooler at 4°C for a day and later dried at 105°C for 24 hours. The dried bones were thereafter placed in dessicators. The samples were analyzed for breaking strengths using a shear force measurement as outlined by Wilson

(1991). The bone morphometry parameters taken included the following; the shank weight, shank length, shank width, robusticity index, bone breaking strength and bone density. The robusticity index and bone breaking strength were calculated as follows:

$$\text{Robusticity index} = \text{Bone length} / (\text{bone weight})^{1/3}$$

$$\text{Bone breaking strength (N/mm}^2\text{)} = \text{Force/Area}$$

Statistical design and analysis

The experiment was arranged in a 2 × 2 factorial layout and data collected were subjected to completely randomized design. Significant ($p < 0.05$) differences among treatment means were separated using Duncan's Multiple Range Test as contained in SAS (2003) package. The statistical model in the factorial experimental layout is shown below:

$$Y_{ijk} = \mu + H_i + B_j + (HB)_{ij} + \varepsilon_{ijk}$$

Where,

Y_{ijk} = individual observation of dependent variable (output)

μ = population mean

H_i = effect of the i th housing systems (i = Deep litter and Outdoor run)

B_j = effect of the j th breed (j = Arbor Acre and Marshall)

$(HB)_{ij}$ = effect of interaction between housing and breed

ε_{ijk} = random error associated with each observation

Table 1: Main effects of breed and housing system on the growth performance of broiler chickens

Parameter	Breed		SEM	Housing system		SEM
	Marshall	Arbor Acre		Deep litter	Outdoor run	
Initial weight (g/b)	602.33	610.00	15.82	587.00	580.33	21.64
Final weight (g/b)	1890.15 ^b	2012.06 ^a	20.07	1900.00	1850.16	48.10
Weight gain (g/b/d)	45.82 ^b	48.45 ^a	2.58	49.70 ^a	49.11 ^b	3.44
Feed intake (g/b/d)	144.60 ^b	151.74 ^a	6.17	144.92 ^b	147.89 ^a	7.09
FCR	3.16 ^a	3.13 ^b	0.07	2.92 ^b	3.01 ^a	0.20
Mortality (%)	0.00	0.00	0.00	0.00	0.00	0.00

^{a,b}: Means on the same row with different superscripts are significantly ($p < 0.05$) different

SEM: Standard Error of Mean

FCR: Feed Conversion Ratio

Table 2: Interaction effects between breed and housing system on the growth performance of broiler chickens

Housing system Parameter	Marshall		Arbor Acre		SEM
	Deep litter	Outdoor run	Deep litter	Outdoor run	
Initial weight (g/b)	649.00	656.67	660.00	660.00	23.60
Final weight (g/b)	1850.34	1814.33	2083.33	1916.67	51.86
Weight gain (g/b/d)	47.38 ^{ab}	46.55 ^b	50.02 ^a	44.88 ^c	3.89
Feed intake (g/b/d)	144.72 ^b	147.06 ^a	143.26 ^b	145.12 ^{ab}	7.56
FCR	3.05 ^b	3.16 ^{ab}	2.86 ^c	3.23 ^a	0.16
Mortality (%)	0.00	0.00	0.00	0.00	0.00

^{a,b,c}: Means on the same row with different superscripts are significantly ($p < 0.05$) different

SEM: Standard Error of Mean

FCR: Feed Conversion Ratio

Results

Effects of breed and housing system on growth performance of broiler chickens

The main effects of breed and housing system on growth performance of broiler chickens are presented in Table 1. Breed significantly ($p<0.05$) affected most of the parameters measured with Arbor Acre breed having higher final weight (2012.06g/b), weight gain (48.45g/b/d) and feed intake (151.74g/b/d) than the final weight (1890.15g/b), weight gain (45.82g/b/d) and feed intake (144.60g/b/d) recorded for Marshall breed. Arbor Acre breed also had a better feed conversion ratio (FCR) of 3.13 than Marshall breed which had a poorer FCR (3.16). Birds reared on deep litter housing system recorded significantly ($p<0.05$) higher weight gain (49.70g/b/d) and a better FCR of 2.92 while those managed on outdoor run consumed more feed (147.89g/b/d).

The weight gain, feed intake and feed conversion ratio were significantly ($p<0.05$) affected by the interaction between breed and housing system (Table 2). The highest weight gain (50.02g/b/d) was obtained in Arbor Acre breed reared on deep litter housing system while the lowest weight gain (44.88g/b/d) was obtained in Arbor Acre breed reared on outdoor run. Correspondingly, the feed intake and feed conversion ratio were lowest (143.26g/b/d) and best (2.86) in Arbor Acre breed of broiler chickens reared on deep litter housing system.

Effects of breed and housing system on carcass traits of broiler chickens

Table 3 showed the main effects of breed and housing system on carcass traits of broiler chickens. The results of carcass traits showed significantly ($p<0.05$) higher plucked weight (1721.28g) and eviscerated weight (1373.06g) in Arbor Acre breed compared with

Table 3: Main effect of breed and housing system on carcass traits of broiler chickens

Parameter	Breed			Housing system		
	Marshall	Arbor Acre	SEM	Deep litter	Outdoor run	SEM
Live weight (g)	1884.66	1912.89	41.73	1856.50	1820.67	50.21
Plucked weight (g)	1691.46 ^b	1721.28 ^a	42.72	1675.34 ^a	1594.67 ^b	51.76
Eviscerated weight (g)	1342.98 ^b	1373.06 ^a	51.06	1350.83 ^a	1300.17 ^b	50.88
Dressing percent (%)	71.26	71.78	1.98	72.78	71.41	2.91
Cut-up parts¹						
Thigh	10.68	10.95	0.24	11.05	10.59	0.22
Drumstick	11.81	11.85	0.27	12.33	11.60	0.30
Breast	22.00	22.40	1.02	22.80 ^a	22.52 ^b	1.06
Back	13.60	13.53	0.73	13.41	13.51	0.81
Wings	9.45	9.51	0.63	8.54	8.73	0.63
Neck	5.52	5.60	0.25	5.84	5.50	0.28
Abdominal fat	0.57	0.60	0.06	2.09 ^a	0.79 ^b	0.09
Organs²						
Heart	0.49	0.42	0.03	0.43	0.50	0.02
Lungs	0.60	0.60	0.03	0.58	0.64	0.02
Liver	2.06	1.76	0.10	1.92	1.97	0.09
Spleen	0.14	0.10	0.01	0.12	0.10	0.01
Gizzard	3.00	3.15	0.15	3.45 ^b	3.72 ^a	0.17
Gastro-intestinal tract	5.34	5.41	0.29	4.99	5.10	0.33

^{a,b}: Means on the same row with different superscripts are significantly ($p<0.05$) different

^{1,2}: Expressed as percentages of the live weight

SEM: Standard Error of Mean

Table 4: Interaction effects between breed and housing system on carcass traits of broiler chickens

Breed Housing system Parameter	Marshall		Arbor Acre		SEM
	Deep litter	Outdoor run	Deep litter	Outdoor run	
Live weight (g)	1860.26	1840.91	1930.67	1914.88	30.70
Plucked weight (g)	1650.67	1640.71	1732.41	1710.67	23.15
Eviscerated weight (g)	1318.34	1300.26	1373.33	1360.45	22.89
Dressing percent (%)	70.86	70.66	71.13	71.05	3.09
Cut-up parts¹					
Thigh	10.50 ^{ab}	10.25 ^b	11.02 ^a	10.33 ^b	0.24
Drumstick	11.01 ^{ab}	10.85 ^b	12.11 ^a	11.47 ^b	0.28
Breast	22.95 ^b	22.11 ^c	23.05 ^a	22.65 ^b	1.09
Back	13.69	12.51	13.84	13.00	0.65
Wings	9.26	9.18	10.20	9.72	0.25
Neck	5.53	5.53	6.14	5.06	0.30
Head	3.36	3.50	3.68	3.71	0.39
Shank	5.53	5.53	6.14	5.06	0.31
Abdominal fat	1.85 ^a	0.52 ^c	1.96 ^a	1.00 ^b	0.09
Organs²					
Heart	0.47	0.51	0.48	0.46	0.05
Lungs	0.56	0.64	0.60	0.52	0.04
Liver	2.09	2.02	1.74	1.77	0.12
Spleen	0.15	0.14	0.10	0.10	0.02
Gizzard	3.10 ^c	3.19 ^b	3.18 ^{ab}	3.38 ^a	0.16
Gastro-intestinal tract	4.83 ^c	5.85 ^a	5.15 ^b	5.68 ^b	0.37

^{a,b,c}: Means on the same row with different superscripts are significantly ($p < 0.05$) different

SEM: Standard Error of Mean

^{1,2}: Expressed as percentages of the live weight

Marshall breed which had lower plucked weight (1691.46g) and eviscerated weight (1342.98g). Breed had no significant ($p > 0.05$) influence on the dressing percentage, cut-up parts and organs of broiler chickens. The housing system had significant ($p < 0.05$) effect on plucked weight and eviscerated weight with birds kept on deep litter having 1675.34g and 1594.67g, respectively while those reared on outdoor run recorded 1350.83g and 1300.17g for plucked weight and eviscerated weight, respectively. Birds reared on deep litter housing system obtained higher value of 22.80% for breast, while those on outdoor run recorded a lower value of 22.52%. Also, broiler chickens reared on deep litter recorded higher abdominal fat (2.09%) compared with those managed on outdoor run (0.79%). It was observed that birds

managed on outdoor run had a higher gizzard (3.72%) compared to those kept on deep litter (3.45%).

Table 4 showed the effects of interaction between breed and housing system on carcass traits of broiler chickens. Arbor Acre breed of broiler chickens reared on deep litter housing system recorded the highest ($p < 0.05$) value for thigh (11.02%), drumstick (12.11%), breast (23.05%), abdominal fat (1.96%) and gizzard (3.38%). Arbor Acre on outdoor run recorded the highest value of 3.38 for gizzard. Also, Marshall breed managed on outdoor run had the highest gastro-intestinal tract (5.85%), compared to those reared on deep litter housing system with the least (4.83%) gastro-intestinal tract.

Effects of breed and housing system on bone morphometry of broiler chickens

The main effects of bone morphometry of broiler chickens as affected by breed and housing system is as shown in Table 5. Breed and housing system had no significant ($p>0.05$) effect on all the parameters measured with the exception of bone breaking strength.

There was significant ($p<0.05$) increase in bone breaking strength from 10.45N/mm² in Marshall breed to 10.68 N/mm² in Arbor Acre breed of broiler chickens. Also, birds reared on outdoor run had greater bone strength (14.61 N/mm²) compared with those kept on deep litter housing system (10.68 N/mm²).

Table 5: Main effects of breed and housing system on bone morphometry of broiler chickens

Parameter	Breed			Housing system		
	Marshall	Arbor Acre	SEM	Deep litter	Outdoor run	SEM
Shank weight (g)	2.63	2.94	0.15	2.94	3.03	0.26
Shank length (mm)	3.17	2.52	0.32	2.52	2.57	0.39
Shank width (mm)	0.44	0.56	0.10	0.56	0.43	0.11
Robusticity index	2.31	1.84	0.21	1.84	1.87	0.30
BBS (N/mm ²)	10.45 ^b	10.68 ^a	1.08	10.68 ^b	14.61 ^a	1.01
Bone density (g/mm ³)	0.57	0.57	0.03	0.57	0.61	0.03

^{a,b}: Means on the same row with different superscripts are significantly ($p<0.05$) different

SEM: Standard Error of Mean

BBS: Bone breaking strength

Table 6: Interaction effect between breed and housing system on bone morphometry of broiler chickens

Housing system Parameter	Breed Marshall		Breed Arbor Acre		SEM
	Deep litter	Outdoor run	Deep litter	Outdoor run	
Shank weight (g)	2.43 ^b	2.83 ^{ab}	3.45 ^a	3.23 ^a	0.20
Shank length (mm)	3.26 ^a	3.09 ^a	1.78 ^b	2.05 ^{ab}	0.54
Shank width (mm)	0.33 ^b	0.55 ^{ab}	0.79 ^a	0.31 ^b	0.20
Robusticity index	2.41 ^a	2.21 ^b	1.72 ^c	1.53 ^c	0.31
BBS (N/mm ²)	12.09 ^b	20.65 ^b	9.27 ^b	20.41 ^a	1.42
Bone density (g/mm ³)	0.56	0.59	0.59	0.64	0.01

^{a,b}: Means on the same row with different superscripts are significantly ($p<0.05$) different

SEM: Standard Error of Mean

BBS: Bone breaking strength

In the interaction effects between breed and housing system on bone morphometry of broiler chickens (Table 6), the results showed that Arbor Acre on deep litter and outdoor run had higher shank weights (3.45g and 3.23g, respectively), while Marshall breed on deep litter had the lowest shank weight of 2.43g. The shank length of Marshall breed reared on both housing systems was also highest (deep litter- 3.26 mm and outdoor run- 3.09 mm) compared with the lower shank length obtained in Arbor Acre reared on both housing systems (deep litter- 1.78 mm and outdoor run- 2.05 mm).

It was also observed that Marshall and Arbor Acre breeds reared on deep litter obtained higher robusticity index of 2.41 and 1.72, respectively. Also, 20.65 N and 20.41 N were required to break (Bone Breaking Strength) the shank of Marshall and Arbor Acre breeds of broiler chickens reared on outdoor run, respectively. That is, the value of bone breaking strength required for the shank of broiler chickens reared on outdoor run was greater than deep litter.

Discussion

The higher weight gain and better feed conversion ratio observed in broiler chickens reared on deep litter housing system contradict the findings of Santos *et al.* (2005) which demonstrated that the body weight gain of broiler chickens in confined system was lower than those kept in the semi-intensive housing system. Birds reared on outdoor run consumed more feed, but had lower body weight than those reared on deep litter. This could be as a result of increased exercises and exposure to fluctuating temperature which might have made birds on outdoor run dissipate more energy and eventually contributed to more feed consumption unlike those kept on deep litter. This is contrary to the report of Khan *et al.* (2006) who reported that birds that consume more tend to have higher body weight and a better feed conversion ratio. This could also be as a result of the effect of temperature and photoperiod in extensive housing system which has the potential to influence growth mainly by affecting feed intake (Abbas *et al.*, 2008).

Broiler chickens reared on deep litter were superior in carcass yield than those on outdoor run. This is probably due to their better live weights which in turn resulted in higher degree of carcass meatiness (Bartov, 1998 and Agunbiade, 2000). This is in agreement with the observation of Goliomytis *et al.* (2003) that chickens with heavy body weights produce a greater percentage of breast meat per carcass weight. The organs were also significantly affected and this confirmed the observation of Onimisi *et al.* (2008) which stated that the difference in gizzard weight seems to correlate with feed consumption. This also agreed with the findings of Esonu *et al.* (2008) who reported organ weight as an index of nutrients retained by the birds. The increase in feed consumption in birds raised on outdoor run tends to enlarge the gizzard capacity to enable them cope with higher volume of feed. The accumulation of abdominal fat observed in birds reared on deep litter was probably due to restricted movement in confined housing system.

Bones play an essential role in support

of the body mass and protection of internal organs. The housing systems had effect on the bone strength of broiler chickens. The bone of birds reared on outdoor run was stronger compared with those on deep litter. This may be due to comfort movement and bumping of the shank in the outdoor run as a result of availability of more space for exercises thereby making leg muscles stronger (Shields *et al.*, 2004). Dynamic loading occurs during normal movement and causes stress and strain to bone and muscle that keep the skeletal system healthy. It was also noted that under natural conditions, birds consume little stones (calcium carbonate and calcium silicate), which are important for digestion as well as the formation of skeleton. Thus, lack of exercise or reduced locomotion activities in confinement leads to bone fragility and impaired bone strength as observed in birds kept on deep litter.

Conclusion

The study concluded as follows:

- The growth performance of the two breeds reared on deep litter was better in terms of weight gain and feed conversion ratio than in the outdoor run. However, Arbor acre breed recorded better performance than Marshall breed.
- Broiler chickens reared on deep litter recorded higher carcass yield compared to those on outdoor run. However, birds reared on outdoor run had higher bone strength than those kept on deep litter housing system.
- Arbor Acre breed of broiler chickens could be reared on deep litter housing system for better performance. However, outdoor run could be adopted to enhance better bone strength in both breeds of broiler chickens.

Impact

The study emphasized the relevance of management systems on the production of broiler chickens in order to bridge the ever-widening gap of meat supply in human food. It further lend credence to the limitation of

outdoor run for profitable broiler chickens production.

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RECORDS OF CAESAREAN SECTIONS IN BITCHES PRESENTED TO THE VETERINARY TEACHING HOSPITAL, FEDERAL UNIVERSITY OF AGRICULTURE, ABEOKUTA, NIGERIA

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Abstract

Case records at the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta were reviewed between January, 2012 and December, 2014 to determine the frequency of caesarean sections (CS) performed in dogs. Data recorded include breed and age of dogs, indication for CS and method of anaesthesia used. Other records obtained were ultrasound information regarding fetal viability prior to surgery, and surgical outcome three days post operation. Data were presented using descriptive statistics. Association between the time of presentation of bitches for CS, method of anaesthesia and surgical outcome was determined using chi square test. A p value ≤ 0.05 was considered significant. Sixteen (16) CS were performed during the study period. Breeds involved were Boerboel, Rottweiler, Alsatian, bulldog, Lhasa apso and mongrel dogs, with the highest record in Boerboel. Mean age of the dogs was 3.1 ± 1.2 years. Eleven (11) of the dogs were nulliparous; while five (5) had previous normal parturitions. Fifteen (15) dogs had emergency CS, while one (1) had elective CS. Indication for CS in the dogs included uterine inertia (13), uterine torsion (1) and vaginal prolapse (1). Mean time of presentation of the bitches for CS was 48.6 ± 28.6 hours. Epidural anaesthesia, general anaesthesia with either Diazepam + Propofol combinations or Xylazine+ Ketamine+ Diazepam were used. Surgical outcome were good in thirteen (13) bitches and poor in three (3) bitches, while the outcome on the puppies was good in eight (8) of the caesarean section. Time of presentation of the bitches for CS and anaesthesia type were significantly ($p < 0.05$) associated with surgical outcome in both the bitches and the puppies. It was concluded that CS in dogs are seldomly performed and most cases involved emergency management of dystocia. Duration of labour and type of anaesthesia used are important risk factors for the outcome of CS in both the dam and the puppies.

ETUDE DES DONNÉES DES CÉSARIENNES PRATIQUÉES SUR DES CHIENNES PRÉSENTÉES À L'HÔPITAL UNIVERSITAIRE VÉTÉRINAIRE DE L'UNIVERSITÉ FÉDÉRALE DE L'AGRICULTURE D'ABEOKUTA AU NIGERIA

Résumé

Des fiches de chiennes présentées à l'hôpital universitaire vétérinaire de l'Université fédérale de l'Agriculture d'Abeokuta ont été examinées entre janvier 2012 et décembre 2014, dans le but de déterminer la fréquence des césariennes (CS) pratiquées sur ces animaux. Les données enregistrées comprenaient la race et l'âge des chiennes, les raisons de la césarienne et la méthode d'anesthésie utilisée. Les autres données recueillies étaient des informations obtenues par échographie sur la viabilité du fœtus avant la chirurgie, et le résultat chirurgical trois jours après la césarienne. Les données ont été présentées en utilisant des statistiques descriptives. L'association entre le moment de présentation des chiennes pour césarienne, la méthode d'anesthésie et le résultat chirurgical a été déterminée en utilisant le test du chi carré. Une valeur $p \leq 0,05$ a été considérée comme significative. Seize (16) césariennes avaient été effectuées durant la période couverte par l'étude. Les races ayant subi une césarienne étaient Boerboel, Rottweiler, Alsatian, Bulldog, Lhasa apso ainsi que les chiens bâtards, le plus grand nombre de césariennes ayant été enregistré dans la race Boerboel. L'âge moyen des chiennes était de $3,1 \pm 1,2$ ans. De l'ensemble des chiennes, onze (11) étaient nullipares, tandis que cinq (5) avaient eu des parturitions précédentes.

normales. Quinze (15) chiennes avaient subi une césarienne en urgence, tandis qu'une (1) avait subi une césarienne de convenance. Les raisons du choix de la césarienne comprenaient l'inertie utérine (13), la torsion de l'utérus (1) et le prolapsus vaginal (1). Le moment de présentation des chiennes pour césarienne était de $48,6 \pm 28,6$ heures. L'anesthésie péridurale, l'anesthésie générale avec l'une des combinaisons Diazepam + propofol ou xylazine + kétamine + diazepam ont été utilisées. Le résultat chirurgical était bon chez treize (13) chiennes et faible chez trois (3) chiennes, tandis que le résultat sur les chiots était bon pour huit (8) des chiennes ayant subi une césarienne. Le moment de présentation des chiennes pour césarienne et le type d'anesthésie étaient significativement ($p < 0,05$) associés au résultat de l'intervention chirurgicale aussi bien chez les chiennes que pour les chiots. Il a été conclu que la césarienne est rarement effectuée chez les chiennes et la plupart des cas constituait une prise en charge urgente de la dystocie. La durée du travail et le type d'anesthésie utilisé sont des facteurs de risque importants pour le résultat de la césarienne à la fois pour la chienne et les chiots.

Introduction

Dystocia is defined as difficult birth or inability to expel fetus through the birth canal without assistance (Linde-Forsberg & Persson, 2007). It is a frequent problem in small animal reproduction and the incidence has been estimated to be around 5% in bitches (Max & Jurka, 2013). In Swedish bitches, an overall incidence rate of dystocia was estimated at 5.7 cases/1,000 dog-years at risk (Bergstrom *et al.*, 2006), while the prevalence of dystocia for bitches in Nigeria was estimated as 2.9 percent (Ajala & Fayemi, 2011). In some breeds, parturition disorders are significantly more frequent than in the whole population (Münnich & Küchenmeister, 2009).

Uterine inertia is the most common cause of dystocia in dogs and is being classified as primary or secondary based on whether the myometrial contraction was sufficient enough to result in expulsion of one or some of the puppies (Linde-Forsberg & Persson, 2007). Causes of primary uterine inertia include failure of the uterus to respond to fetal signal because only one or two pups are present (single-pup syndrome), overstretching of the myometrium by large litters, excessive fluid or oversized fetuses, nutritional imbalance, infiltration of myometrium, age-related changes and systemic diseases in the bitch resulting in intra-uterine fetal death (Bergstrom *et al.*, 2006). It is generally admitted, that brachycephalic breeds, such as Bulldogs or Boston terriers, are prone to obstructive dystocia (Linde-Forsberg & Persson, 2007).

Traditionally, the total length of parturition and the time required for puppy expulsion are considered as the most

important parameters affecting neonatal viability (Groppetti *et al.*, 2010). Control of the delivery process should be recommended when labour is delayed in order to decrease peri-parturient puppy mortality. These include administration of oxytocin and prostaglandins to increase myometrial contraction (Guyton & Hall, 2000), digital manipulation using forceps (Max and Jurka, 2013) and surgical delivery of the puppies through caesarean section (Evans & Adams, 2010). In a previous study, obstetrical manipulations and/or medical treatment was successful only in 27.6% of cases of dystocia in dogs, while surgical intervention was required in approximately 60- 80% of dystocia cases in the bitches and queens (Bergstrom *et al.*, 2006; Traas, 2008).

Caesarean rate in humans have been reported to be approximately 24% of all births in England and 31.8 % of all birth in the USA (Hamilton *et al.*, 2008). However, statistics on caesarean sections in dogs are difficult to come by (Evans & Adams, 2010). Some breeds of dogs such as Boston terrier, Bull dogs have been reported to have high rate of caesarean section (Bergstrom *et al.*, 2006; Evans & Adams, 2010). Previous reports showed that 37.5% of bitches with dystocia in Nigeria were managed with caesarean section (Ajala & Fayemi, 2011). However, there are no records of caesarean section in bitches in Nigeria. This preliminary study provides a record of caesarean section in bitches presented to the Veterinary Teaching Hospital, FUNAAB following elective request from the owner or dystocia. This study also evaluated the effect of the time of presentation of the bitches for caesarean section and the type of anaesthesia used, on the outcome of the dam and neonatal survivability of the delivered puppies.

Methodology

Records of caesarean operation performed in bitches at the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta between January, 2012 and December, 2014 were reviewed. Data recorded were the breed and age of the dog, the number of times of previous parturition prior to the caesarean section, the time duration from onset of parturition or observation of greenish vagina discharge to the time of presentation of the bitch for caesarean surgery, the indication for caesarean surgery and method of anaesthesia used. Other records obtained were the ultrasound information regarding the number and viability of the fetuses, the number and state of puppies delivered, as well as the outcome of the surgery on the dam and the puppies three days post operation.

The data were presented using descriptive statistics. The association between the time of presentation of the bitches, method of anaesthesia used and surgical outcome for the dam and the puppies was determined using chi square test. A p value ≤ 0.05 was considered significant.

Results

Sixteen (16) cases of caesarean section were performed at the Veterinary Teaching Hospital, FUNAAB between the study periods (Table 1). These cases were recorded in Boerboel, Rottweiler, Alsatian, Bull dog, Lhasa apso and Mongrel dogs with the highest prevalence in the Boerboel dogs (Fig. 1). The mean age of the dogs was 3.1 ± 1.2 years (Age range is 1.5 to 6 years). Eleven (11) of the dogs presented for caesarean section were nulliparous, while five (5) of the dogs have had previous normal parturitions before the caesarean section (Fig. 2). Fifteen (15) of the dogs had emergency caesarean section, while caesarean section was performed in one (1) of the dogs on elective basis (Fig. 3). Emergency caesarean sections were performed secondary to uterine inertia (13), uterine torsion (1) and vaginal prolapse (1) (Fig. 3). The causes of uterine inertia in the dogs were classified as singleton pregnancy (1), intrauterine fetal death

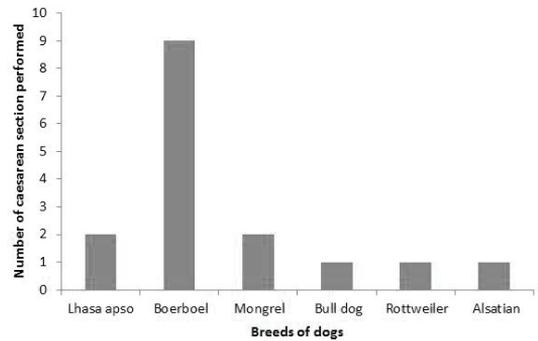


Figure 1: Breed distribution of caesarean section performed at the Veterinary Teaching Hospital, FUNAAB

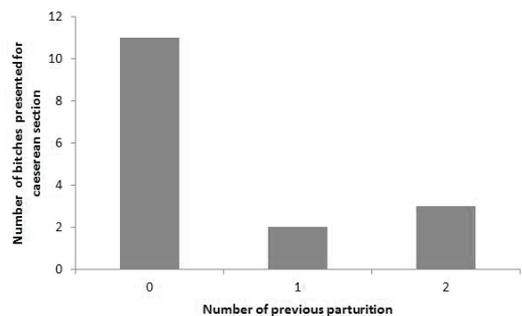


Figure 2: Number of previous parturition of dogs presented for caesarean section at the Veterinary Teaching Hospital, FUNAAB

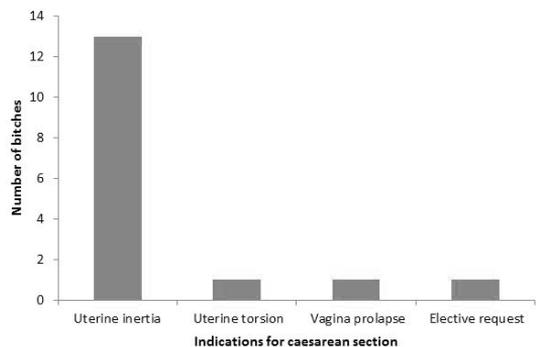


Figure 3: Indications for performing caesarean section in dogs presented at the Veterinary Teaching Hospital, FUNAAB

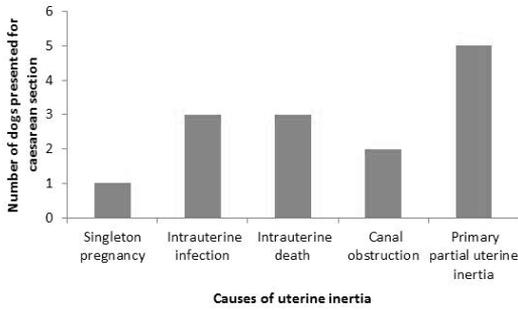


Figure 4: Causes of uterine inertia in dogs presented for caesarean section at the Veterinary Teaching Hospital, FUNAAB

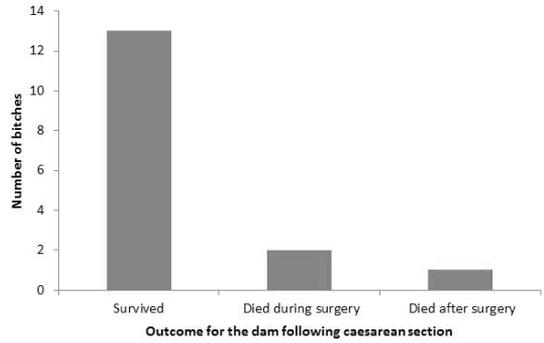


Figure 7: Surgical outcome of dam presented for caesarean section at the Veterinary Teaching Hospital, FUNAAB

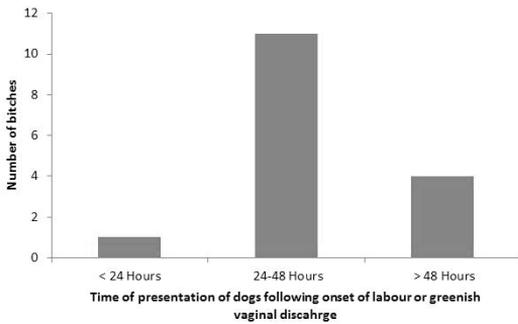


Figure 5: Time of presentation at the hospital following onset of labour or greenish vaginal discharge of dogs presented for caesarean section at the Veterinary Teaching Hospital, FUNAAB

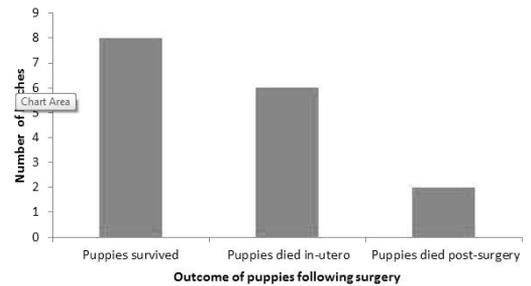


Figure 8: Outcome of puppies of bitches presented for caesarean section at the Veterinary Teaching Hospital, FUNAAB

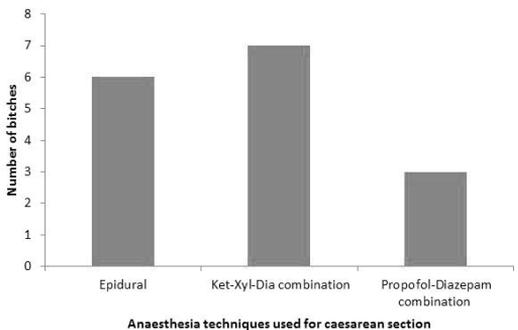


Figure 6: Anaesthesia techniques used for dogs presented for caesarean section at the Veterinary Teaching Hospital, FUNAAB

(3), intra-uterine fetal infection or placenta separation (3), canal obstruction (2) and primary incomplete uterine inertia (5) (Fig. 4). The mean duration of presentation of the bitches at the hospital was 48.6 ± 28.6 hours. Twelve (12) of the dogs in which emergency caesarean section were performed were presented between 24-48 hours after the onset of labour or after greenish vagina discharge was observed (Fig. 5). Epidural injections of lignocaine or general anaesthesia with either Diazepam + Propofol combinations or Xylazine+ Ketamine+ Diazepam were the recorded methods of anaesthesia for the caesarean sections in the bitches (Fig.6). Surgical outcome for the dam was described as good in thirteen (13) bitches and poor in three (3) bitches (Fig. 7), while the outcome on the puppies was good in eight (8) of the caesarean section (Fig. 8).

Time of presentation of the bitches for caesarean section and the type of anaesthesia were significantly ($p < 0.05$) associated with surgical outcome in both the bitches and the puppies.

Table 1: Record of caesarean section in dogs presented at the Veterinary Teaching Hospital, FUNAAB

S/N	Breed	Age (Yrs)	Number of previous parturition	Indication for Caesarean section	Time of Presentation	Method of Anaesthesia	State of fetus prior to C/S	Number of puppies delivered	Outcome of C/S
1	Boerboel	2	0	1. Greenish vagina discharge on day 63 post mating. 2. Primary uterine inertia secondary to uterine torsion	72 hours after onset of vaginal discharge	Lumbosacral epidural anaesthesia with 2% Lignocaine	Two fetuses without fetal beat and one with fetal beat on ultrasound	One live and two dead puppies	Both Dam and live fetus survived
2	Boerboel	2.5	0	Secondary uterine inertia with uterine infection	36 hours after onset of labour	General anaesthesia with Ketamine, xylazine and diazepam	Several fetuses without fetal heart beat and two fetuses with fetal heart beat	Three live and eight dead puppies	Dam survived but live puppies died few hours after C/S
3	Lhasa apso	1.5	0	Greenish vagina discharge on day 62 post mating	28 hours after onset of vaginal discharge	General anaesthesia with Ketamine, xylazine and diazepam	One fetus with reduced fetal beat	One live puppy	Both dam and puppies survived.
4	Boerboel	6	2	Primary uterine inertia with intra-uterine fetal death	48 hours after onset of labour	Lumbosacral epidural anaesthesia with 2% Lignocaine	Multiple fetuses without fetal heart beat or fetal movement	Seven dead fetuses	Dam survived

S/N	Breed	Age (Yrs)	Number of previous parturition	Indication for Caesarean section	Time of Presentation	Method of Anaesthesia	State of fetus prior to C/S	Number of puppies delivered	Outcome of C/S
5	Boerboel	4	0	Primary uterine inertia	36 hours after onset of labour	Lumbosacral epidural anaesthesia with 2% Lignocaine	Multiple fetuses with fetal heart beat	Six live fetuses	Both dam and fetuses survived
6	Boerboel	5	1	Primary uterine inertia	28 hours after onset of labour	Lumbosacral epidural anaesthesia with 2% Lignocaine	Multiple fetuses with fetal heart beat	Five live fetuses	Both dam and fetuses survived
7	Lhasa apso	4	2	Primary uterine inertia due to obstruction of birth canal by dead fetus	48 hours after onset of labour	General anaesthesia with Ketamine, xylazine and diazepam	Multiple fetuses with fetal heart beat	Five live fetuses and two	The dam died after surgery but the fetuses survived
8	Mongrel	3	2	Primary uterine inertia and intra-uterine fetal death	96 hours after onset of labor	General anaesthesia with Ketamine, xylazine and diazepam	Multiple fetuses with absence of fetal heart beat and fetal movement	Six dead fetuses	Dam died during surgery
9	British bulldog	2	0	Elective request	48 Hours before the ultrasound estimated delivery date	General anaesthesia with diazepam and Propofol	Several fetuses with fetal heart beat and active fetal movement	Seven live fetuses	Both dam and fetus survived

S/N	Breed	Age (Yrs)	Number of previous parturition	Indication for Caesarean section	Time of Presentation	Method of Anaesthesia	State of fetus prior to C/S	Number of puppies delivered	Outcome of C/S
10	Boerboel	3	0	Primary uterine inertia secondary to vagina prolapse	36 hours after onset of labour	General anaesthesia with diazepam and Propofol	Several fetuses with fetal heart beat	Eleven live fetuses	Both dam and fetuses survived
11	Boerboel	2	0	Greenish vagina discharge by 54 day post mating	28 hours after onset of vaginal discharge	General anaesthesia with diazepam and Propofol	Several fetuses with reduced fetal beat	Six live but immature puppies	Dam survived but puppies died within three days of surgery
12	Rottweiler	3	1	Primary uterine inertia	18 hours after onset of labour	Lumbosacral epidural anaesthesia with 2% Lignocaine	Multiple fetuses with fetal heart beat	Seven live fetuses	Both dam and fetuses survived
13	Boerboel	4	0	Primary uterine inertia	36 hours after onset of labour	General anaesthesia with Ketamine, xylazine and diazepam	Multiple fetuses with fetal heart beat	Six live fetuses	Both dam and fetuses survived
14	Alsatian	2	0	Primary uterine inertia	28 hours after onset of labour	Lumbosacral epidural anaesthesia with 2% Lignocaine	Multiple fetuses with fetal heart beat	Seven live fetuses	Both dam and fetuses survived

S/N	Breed	Age (Yrs)	Number of previous parturition	Indication for Caesarean section	Time of Presentation	Method of Anaesthesia	State of fetus prior to C/S	Number of puppies delivered	Outcome of C/S
15	Mongrel	3	0	Primary uterine inertia due to obstruction of birth canal by dead fetus	96 hours after onset of labour	General anaesthesia with Ketamine, xylazine and diazepam	Multiple fetuses without fetal heart beat	Five dead fetuses	The dam died during surgery
16	Boerboel	3	0	Primary uterine inertia and intra-uterine fetal death	96 hours after onset of labor	General anaesthesia with Ketamine, xylazine and diazepam	Multiple fetuses with absence of fetal heart beat and fetal movement	Six dead fetuses	Dam survived

Discussion

The result of this preliminary study showed that caesarean section was seldomly performed in dogs at the FUNAAB Veterinary Teaching Hospital with an average of 5.3 caesarean operations per year. In addition, Boerboels are the most presented breed for caesarean operation, while emergency caesarean operations were most commonly performed. Also, the result showed that uterine inertia were the major indication for caesarean operations performed at the FUNAAB Veterinary Teaching Hospital, while the duration of presentation of dogs for caesarean section and the type of anaesthesia used significantly influenced the outcome of surgery in both the day and the puppies.

Previous studies have shown that the breeds of dogs commonly undergoing elective caesarean section were the bull-dog, Labrador retriever, Golden retriever and Yorkshire terrier (Moon *et al.*, 1998). The only elective caesarean section in this study was performed in a primiparous bull-dog. On the other hand, bull-dogs, boxers and Scottish terrier were reported among the common breeds of dogs that undergo caesarean surgery on emergency basis. This current report showed that Boerboel dogs accounted for the most of the emergency surgery presented at the FUNAAB Veterinary Teaching Hospital. It is difficult to establish whether the high number of caesarean surgery performed in this breed is a true reflection of high incidence of dystocia in the breed or that the breed was just over-represented. However it is note-worthy that there is high incidence of primary inertia in the breed. Also one of the Boerboel in this study had an emergency caesarean surgery twice as a result of primary uterine inertia.

It had been reported that uterine inertia is the most common cause of dystocia in dogs (Linde-Forsberg & Persson, 2007). Thirteen (81.2%) of the cases of the dystocia cases presented at the FUNAAB Veterinary Teaching Hospital were as a result of primary uterine inertia. This was comprised of conditions such as primary partial uterine inertia, singleton pregnancy, intrauterine infection (premature

placenta separation), intrauterine fetal death and canal obstruction by dead fetus.

Puppies death rate in the neonatal period has been reported to be between 10% and 30% with an average of 12% (Linde-Forsberg & Persson, 2007; Indrebo *et al.*, 2007). Puppies from two of the sixteen bitches presented from surgery died within two hours of delivery by caesarean surgery. Majority of these puppies were not fully active after resuscitation. This might be related to the time lapse between onset of labour and time of presentation of the bitch for surgery, as well as the type of anaesthesia used. Puppies that were delivered from bitches that were presented more than 48 hours after onset of labour were either delivered dead or were difficult to resuscitate. Outcome of caesarean section on puppies delivered have been shown to be dependent of the type of anaesthesia used. Anaesthesia technique incorporating xylazine or methoxyflurane have been associated with poor outcome in the puppies because of excessive fetal depression (Kushnir & Epstein, 2012). Epidural anaesthesia with lignocaine and total intravenous anaesthesia with Propofol resulted in better outcome for the dam and puppies when compared with anaesthesia induced by a combination of xylazine-ketamine and diazepam in the caesarean surgeries performed. The xylazine-ketamine-diazepam combination was associated with severe fetal depression which makes resuscitation more difficult although the combination was cheaper than total intravenous anaesthesia with Propofol.

Finally, two of the bitches presented for caesarean operation died during surgery, while another one died a day following surgery. The three mortality recorded were in bitches presented more than forty eight hours after the onset of labour, while two of them had puppies' already dead in-utero with accompanying severe uterine congestion and infection. Duration of labour before presentation for surgery and the type of anaesthesia used appeared to be important factors for the outcome of caesarean operation in bitches. Bitches are often presented late for caesarean surgery because the owners often wait until all medical

management options have been exhausted in order to avoid the cost of caesarean surgery.

In conclusion, caesarean surgeries in dogs are seldomly performed and mostly involved emergency management of dystocia cases. Duration of labour and type of anaesthesia used are important risk factors for the outcome of caesarean surgery in both the dam and the puppies.

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SOME OBSERVATIONS ON THE COMPARATIVE PERFORMANCE OF TWO 3-ABC ELISAS IN AN AREA OF ENDEMICITY

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Abstract

The performance of Chekit and Priocheck (Ceditest) 3-ABC ELISAs was evaluated in an area of endemicity using 196 sheep and 45 bovine sera. The Checkit ELISA failed to detect 8 positive sheep sera out of 10 detected by the Priocheck ELISA whilst both ELISAs detected 13 bovine sera as positive or borderline. The dissimilarity in the performance of the two ELISAs in testing sheep field sera, a target population, was shown to be consistent with their reported differences following experimental infection in cattle and was largely due to differences in their methodology. Sera expected to form high affinity and small immune complexes are better detected in the system of Priocheck-NS blocking ELISA while sera expected to form low affinity (recent infection) and large immune complexes are better detected in the latter system of Chekit ELISA. Identification of this aspect is crucial for optimization of these assays and for conclusive interpretation of their results.

Prevalence in the test sheep group as detected by the Priocheck ELISA (8.47%) was more pertinent to the expected true prevalence than that detected by the Checkit ELISA (0.51%). The Checkit ELISA could prove to be useful for detection of relatively recent FMD virus circulation and, hence, control plans in enzootic areas.

Key words: Foot-and-mouth disease; 3-ABC serology; Ceditest; Priocheck; Chekit ELISA; Blocking ELISA

QUELQUES OBSERVATIONS SUR LA PERFORMANCE COMPARATIVE DE DEUX ELISA 3-ABC DANS UNE ZONE AUX MALADIES ENDÉMIQUES

Résumé

La performance des tests ELISA 3-ABC Chekit et Priocheck (Ceditest) a été évaluée dans une zone d'endémicité en utilisant 196 sérums ovins et 45 sérums bovins. Le Checkit ELISA n'est pas parvenu à détecter la positivité de 8 sérums ovins, ELISA Priocheck en détecté 10 ; et les deux tests ELISA ont détecté 13 sérums bovins comme étant positifs ou limites. La dissemblance au niveau de la performance des deux ELISA dans les tests des sérums de moutons de terrain, une population cible, correspondait à leurs différences signalées à la suite d'une infection expérimentale des bovins et était largement due à des différences de méthodologie. Les sérums supposés former une forte affinité et de petits complexes immuns sont mieux détectés par le système de l'ELISA de blocage Priocheck-NS, tandis que les sérums censés former une faible affinité (infection récente) et de grands complexes immuns sont mieux détectés par le système d'ELISA CHEKIT. L'identification de cet aspect est cruciale pour l'optimisation de ces tests et pour l'interprétation concluante de leurs résultats.

La prévalence dans le groupe de moutons soumis au test telle que détectée par ELISA Priocheck (8,47%) était plus pertinente pour la prévalence réelle attendue par rapport à celle détectée par ELISA Checkit (0,51%). Le test ELISA Checkit pourrait se révéler utile pour la détection de la circulation relativement récente du virus de la fièvre aphteuse et, par conséquent, pour les plans de contrôle dans les régions enzootiques.

Mots-clés : peste bovine ; sérologie 3-ABC ; Ceditest ; Priocheck ; Chekit ELISA ; ELISA de blocage

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Introduction

Foot-and-mouth disease virus (FMDV) non-structural proteins (NSP) ELISAs are a recent approach in FMD serology. They are developed for the primary purpose of differentiation of infected animals from vaccinated (Berger *et al.* 1990; Sørensen *et al.* 1992; Bergmann *et al.* 1993; De Digo *et al.* 1997; Sørensen *et al.* 1998). These tests are expected to be increasingly used in endemic areas for verification of absence of infection before movement of live animals for international trade (Bronsvort *et al.* 2004) and also like a serosurveillance tool (Bronsvort *et al.* 2008). The OIE has adopted a NSP-ELISA developed by Panaftosa as an index screening method for verification of absence of infection (Brocchi *et al.* 2006). A gold standard test for FMD NSP serology is lacking (Goris *et al.* 2007; Bronsvort *et al.* 2008) but international standard sera for NSP serology of cattle are available and those for sheep and pigs are to follow (OIE, 2008).

A number of NSP ELISAs have been evaluated at different occasions and in different laboratories (Brocchi *et al.* 2006; Kittelberger *et al.* 2008). Their Diagnostic performance characteristics are similar in cattle, sheep and pigs as suggested the data from Panaftosa (OIE, 2008) and the study of Brocchi *et al.* (2006). None of these tests is capable of differentiation FMD infected animals from vaccinated combining 100% diagnostic sensitivity (dSn) with 100% diagnostic specificity (dSp) (Goris *et al.* 2007). Brocchi *et al.* (2006) compared the performance of six NSP-ELISAs; the OIE index test, an IZS-Brescia in-house test and 4 commercial ELISAs (Ceditest which is currently known as Priocheck-NS, Svanovir, Chekit and UBI ELISAs). Sera from negative populations of several European countries and from experimentally infected animals were used to evaluate dSp and dSn respectively. False positive reactors were reported in vaccinated and non-vaccinated cattle. Most of reactors were with borderline values and specificity improved on retesting. On retesting, specificity ranged from 98.3% (OIE index test) to 99.7% (IZS-Brescia in-house test). On the other hand, occurrence of false negative reactors has been confirmed

and two levels of sensitivity were recognized. The more sensitive level was occupied by the OIE index test, Priocheck-NS and the IZS-Brescia ELISAs. The less sensitive ELISAs were the Svanovir, Chekit and UBI ELISAs. Moreover, the dSn values varied according to sampling time post infection. When these ELISAs were used to test field sera, which may represent the target population better than experimentally derived sera, about 40% of results were discordant in sera originating from Zimbabwe and 22% of results were discordant in sera originating from Israel. Not to wrongly estimate the true prevalence when testing field sera (target population) in absence of a reference to a gold standard, statistical analysis is applied (Goris *et al.* 2007; Bronsvort *et al.* 2008) or confirmatory tests are to be used (OIE, 2008).

In this work the performance of two NSP ELISAs, Chekit and Priocheck NS ELISAs was compared and evaluated using cattle and sheep sera from Sudan; an area of endemicity. In Sudan clinical FMD is known in cattle only and serological evidence of infection were obtained in addition from sheep and goats. Moreover, structural protein (SP) serology, often, detected much higher reactors in cattle than in sheep and goats (Abu Elzein *et al.* 1987; Habiela *et al.* 2010; Raouf *et al.* 2012). The presented work takes notice and explains known differences in performance characteristics (dSn and dSp) of the sensitive Priocheck-NS and the less sensitive Chekit ELISA.

Materials and Methods

Serum samples:

Sheep sera were collected from Southern Darfur State in Western Sudan. Sera were originally collected as a part of peste des petits ruminants (PPR) serosurveillance system for assessment of vaccination against PPR, regularly carried by The Veterinary Research Institute (VRI) in Soba-Sudan. Animals were happened to be sampled early in the year 2011 before commencement of the cold season known for FMD outbreaks. Accordingly, these sera were chosen for surveillance of FMD antibodies. A total of 196 sheep sera were used. Together with them, 45 bovine sera,

from the nearby State of Baher Al Ghazal in Southern Sudan, collected during the same period were included in the study for purposes of comparison between the two species. All bovine and ovine species in the study area were of local breeds.

ELISA methods:

The ELISAs compared were the CHEKIT-FMD-3ABC from Bommeli Diagnostics, Bern, Switzerland (Schlach *et al.* 2002) and the Ceditest® FMDV-NS (Cedi Diagnostics B. V., Lelystad, The Netherlands) (Sørensen *et al.* 2005). The Ceditest® FMDV-NS is currently known as Priocheck® FMDV-NS since Cedi Diagnostics Laboratories were acquired by Prionics and re-named the product.

The two tests detect antibodies to the viral non-structural polypeptide 3ABC, expressed as a recombinant antigen in *Escherichia coli* (Chekit ELISA) or in Baculovirus (Priocheck NS). The purified antigen is directly coated to microplates in Chekit ELISA, while in Priocheck-NS it is trapped by a monoclonal antibody. The Priocheck-NS is a blocking ELISA uses an antigen-specific monoclonal horseradish peroxidase conjugate, while the Chekit ELISA is an indirect ELISA uses an anti-species conjugate (monoclonal anti-ruminant-IgG horseradish peroxidase conjugate). Antibodies specific to the 3ABC protein, that may be present in the tested serum sample, will bind to the 3ABC protein and block binding of the conjugate to the antigen in Priocheck-NS or bind the anti-species conjugate in Chekit ELISA. In both ELISAs, serum samples were tested in a single well each, at dilutions 1/5 in Priocheck-NS or 1/100 in Chekit ELISA. The two tests procedures are in 100 µl volume. All procedures, calculations and result interpretations were done as described by the manufacturers.

Chekit ELISA:

Briefly, 100 µl of pre-diluted serum sample was transferred to each well. Each plate contained one well for control positive and another well for control negative sera. After incubation (37 °C for one hour) and washing, conjugate was added and plates incubated for another hour at 37 °C. The chromogen

substrate solution, tetramethyle benzidine (TMB substrate), was added after washing and plates were incubated for 15 minutes at room temperature. The reaction was stopped by addition of the Chekit stop solution. Optical density values were read at 450 nm using Elx808 plate reader (BIO-TEC). The OD values of the positive and negative controls should not exceed 2.0 and 0.5 respectively. The difference between the two values must be ≥ 0.40 . Result was expressed as percentage positivity (PP) value calculated as follow:

$$PP\% = (OD_{\text{sample}} - OD_{\text{negative}} / OD_{\text{positive}} - OD_{\text{negative}}) \times 100$$

Samples with PP value $\geq 30\%$ were considered positive, between 20% and 30% were suspicious and values $< 20\%$ were considered negative.

Priocheck-NS:

Briefly, 1/5 dilution of tested samples, negative, positive and weak positive controls were prepared in respective wells. Only controls were tested at duplicate wells. Plates were sealed and incubated overnight (16-18 hours) at $22 \pm 3^\circ\text{C}$. On the second day, plates were washed and 100 µl of diluted conjugate was dispensed to all wells and incubated for one hour at $22 \pm 3^\circ\text{C}$. The TMB substrate solution was added after washing and plates were incubated at $22 \pm 3^\circ\text{C}$. The reaction was stopped 20 minutes later by addition of a stop solution. The OD values were measured at 450 nm using Elx808 plate reader (BIO-TEC). The average OD value of the two negative control wells was calculated (OD_{450 max}).

Results were expressed as percentage inhibition (PI) values calculated according to the equation:

$$PI = 100 - \frac{\{OD_{450 \text{ test sample}}\} \times 100}{OD_{450 \text{ max}}}$$

For validation of results, the OD_{450 max} must be > 1.0 , the mean PI of the weak positive control $> 50\%$ and the mean PI of the positive control $> 70\%$. Test PI values $< 50\%$ were negative and $\geq 50\%$ were positive.

Quantitative assay:

In each ELISA, similar to the respective screening assay except that sera were tested at dilutions shown in the text.

Results

Performance of the two ELISAs in screening sheep and cattle sera:

The whole lot of sheep sera (196) was tested for 3-ABC antibody using the Chekit ELISA. The assay detected one positive and two borderline sera (0.51%). When 118 of these sera were tested with the Priocheck-NS, 10 sera (8.47%) scored positive (Table 1). On the other hand, when 45 bovine sera were tested with the Chekit ELISA, twelve sera were found to be positive and one showed borderline value and all 13 sera showed positive result with the Priocheck-NS. On other words, the Chekit ELISA failed to detect 9 Priocheck-NS positive sheep sera out of 10 but detected 12 Priocheck-NS positive bovine sera out of 12.

This apparent inability of the Chekit ELISA to detect Priocheck-NS positive sheep sera was investigated by examining PI values in Priocheck-NS of all positive sera. No inconsistency between positive bovine and positive sheep sera could be detected (Table 2). Positive sheep sera that failed to be detected by the Chekit ELISA were all weak positive sera by Priocheck-NS (below 70% PI value) while positive bovine sera (positive in the two tests) showed higher than 70% PI values in Priocheck-NS.

Relationship between positive values of PP in Chekit ELISA and PI in Priocheck NS:

All Chekit positive sera showed in Priocheck-NS (Table 2) PI values above 70%. Results presented in Fig (1) and in Table (2) also show that sera with medium PP in Chekit (above 40% and less than 70%) showed the highest positive blocking activity in Priocheck-NS followed by sera with the lowest positive values in Chekit ELISA (between 30% and 40%). The lowest blocking activity in Priocheck-NS is shown by sera with the highest PP (above 70%) in Chekit ELISA.

Quantitative assay:

Borderline and negative sera by Chekit ELISA that scored positive in Priocheck-NS were further investigated by titration in Chekit ELISA in more than one occasion. Two out of three titrated sera (borderline or negative at 1/100) showed positive and borderline results respectively at the serum dilution of 1/75 (Fig. 2). Moreover, their titration curves displayed similar characteristics; they showed somewhat slow decline between 1/100 and 1/125. The third serum (No. O71) showed a completely reversed titration curve (Fig. 3); it showed a strong positivity of 76.6% at dilution 1/125 but low PP at dilutions 1/100 and 1/75. In comparison, titration curves of screened positive sera (at dilution 1/100) showed sharp decline between 1/100 and 1/125 (Fig. 2). On the other hand, sera screened negative by the two tests remained negative even at dilution 1/50.

When some of these sera were titrated in Priocheck-NS, they showed good titration but sharper decline than stronger and marginally positive/negative sera (Fig. 4). Characteristically, serum No O71 showed, in different trials, the sharpest decline among the test group (Fig 5). That was particularly distinguishable as titration of sera in Priocheck-NS was generally of low depth (Fig. 6).

Discussion

It is generally accepted that the performance of 3-ABC ELISAs is similar in bovine and ovine while differences in dSe between 3-ABC ELISAs have been confirmed (Brocchi *et al.* 2006; Goris *et al.* 2007). In this respect, no discrepancy was detected since sheep sera that failed to be detected as positive by the less sensitive Chekit ELISA were all weak positive by the more sensitive Priocheck-NS (Table 2). Moreover, in this work the failure, exclusively of weak positive Priocheck-NS sera to be detected by the Chekit ELISA, the detected relationship between positive PP values (in Chekit ELISA) and positive PI values (in Priocheck-NS) together with the results of the quantitative assay could suggest a certain pattern for differences in the performance of the two ELISAs.

Table 1: Performance of the two ELISAs in testing sheep sera

Assay type	Checkit	Priocheck	
		+	-
Results	+	1	1
	BL	2	1
	-ve	193	8
Totals		196	10
			108

BL= border line

Table 2: Positive sera (bovine and ovine) by Priocheck-NS and their corresponding PP in Checkit

No. of sera	Species	Checkit :	Value (PP)*	Priocheck (PI)*			
82	Bovine	Positive	40.6%	95.12%			
F93			51%	92.5%			
FI04			58%	89.1%			
F803su			38.26%	88%			
F84			32%	87.6%			
F228su			186.73%	87.28%			
F201su			73.37%	82%			
F85			Borderline	20.5%	64.5%		
Ao1123			Ovine	Positive	37.6%	88%	
Ao1129					Borderline	26.28%	62.37%
Ao801					Negative	Below 20%	67%
O71							62%
Ao864							60%
Ao1139							57%
Do626					52%		
Ao1122					52%		
Ao1148			49.8%				

*Average of 2 or 3 trials in many instances

Both ELISAs detect one biological phenomenon; antibodies to 3-ABC polyproteins. The Priocheck-NS detects serum blocking activity while the Chekit ELISA is an indirect ELISA detecting complexed antibodies. When positivity in the two ELISAs was compared, it was evident that serum blocking activity of positive sera in Priocheck-NS did not correspond with their positivity in Chekit ELISA (Fig 1 and Table 2). It has been shown before that formation of low affinity or large immune complexes decrease serum blocking activity to FMD structural proteins (Raouf *et al.* 2006, 2007) in the system of the LPBE (Hamblin *et al.* 1986). Large and low affinity immune

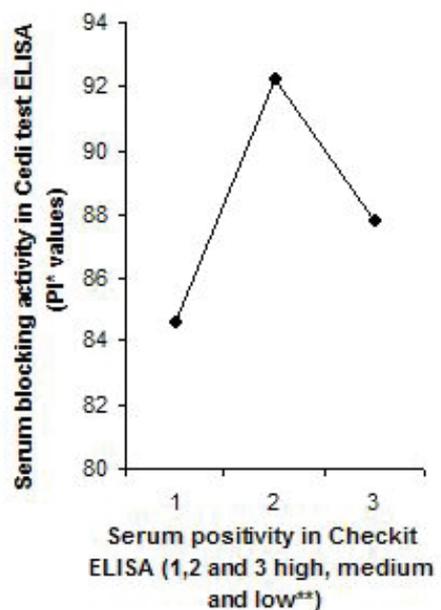
complexes have numerous epitopes which would not be efficiently blocked in comparison to small and good affinity immune complexes and consequently likely to decrease detected blocking activity. Conversely, since the Chekit ELISA detects complexed antibodies, it is to be expected that sera forming large or low affinity immune complexes would show high positivity in the Chekit ELISA as these immune complexes also involve numerous antibodies. Low affinity and large immune complexes form when serum is of low avidity or when antigen/antibody ratio is about equal. It is true that sera are tested at highly different dilutions in the two tests, 1/100 and 1/5, but antigen concentrations also differ

between the two ELISAs. Accordingly, it was not inconsequent that sera with the highest PP in Chekit ELISA would show the lowest blocking activity in Priocheck-NS. Also, it was not inconsequent that sera with the highest blocking activity in Priocheck-NS would show a medium PP in Chekit ELISA. In accord with this justification the finding of Brocchi *et al.* (2006) that the Chekit ELISA is one of the more sensitive assays for detection of early antibody. Early antibodies are expected to form low affinity immune complexes.

One finding in this work was that no Priocheck-NS negative serum was detected positive by Chekit ELISA. On the other hand, part of the discordant results in post outbreak surveillance reported by Brocchi *et al.* (2006) was that 9 sera from Israel and 3 from Zimbabwe were detected positive by the Chekit but not by the 3 sensitive ELISA including the Priocheck-NS (Goris *et al.* 2007). In Israel samples were collected between 30 and 80 days after outbreaks, in Zimbabwe 1 to 5 months after outbreaks and in this work at least 6 months after the known season of FMD outbreaks. It was obvious that, not merely dSn values varied, but the occurrence of this category of reactors (Priocheck-NS negative Chekit positive) decreased, according to sampling time post infection. The disappearance of this category of reactors coincided with the expected disappearance of early antibodies which form low affinity or large immune complexes. The realization of this difference in methodology permits explanation of these findings more than mere discordant results but as a reflection of the epidemiological status in the field (earlier infection).

The whole discordant result in this work was that none of 9 Priocheck-NS positive sheep sera were detected positive by the Chekit ELISA. Similarly, the highest category of discordant results in post outbreak surveillance in Zimbabwe (where sampling time was 1 to 5 months after outbreaks) was 22 bovine sera detected negative by the Chekit but positive by other ELISAs including Priocheck-NS and the two ELISAs with sensitivity level similar to that of the Chekit (Goris *et al.* 2007). It has been verified experimentally in cattle that the

Chekit ELISA became increasingly less sensitive compared to other ELISAs including Ceditest at detection of antibodies present after 28 days from infection and still more so after 100 days (Brocchi *et al.* 2006). In this work, when some of these sheep sera were titrated using Chekit ELISA, they produced positive or borderline results at higher serum concentrations (dilution 1/75). Titration curves of these sera in both ELISAs showed relatively good correlation between serum dilutions and detected activity (Fig. 2 and 4) which could be indicative of their relatively good avidity (Growther, 2001). Their titration curves in Priocheck-NS (Fig. 4 and 6) showed sharper decline than titration curves of other positive and negative sera what established together with their weak positivity signal their lower content of the reacting component. The titration curves of these sera (Fig. 2) and those in Fig. (7) were similar in showing no end points or slow decline at ends of curves. However, it turned out that in both systems (Chekit and LPBE type "SAT I") each group of sera produced positive reaction or higher titer when serum

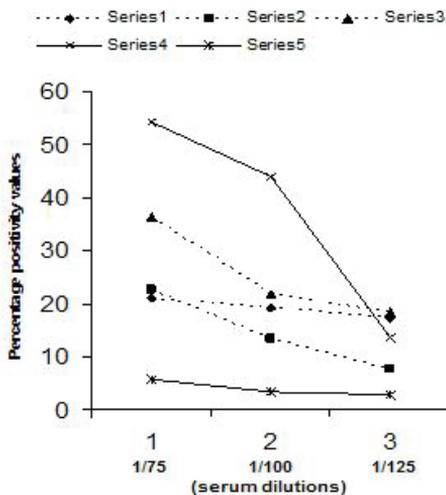


* Percentage inhibition values

** Percentage positivity: 1 (above 70%), 2 (between 40% and 70%) and 3 (less than 40%) (test threshold at 30%)

Figure 1: Relationship between positive values of percentage positivity in Chekit and percentage inhibition in Priocheck.

(Chekit) or antigen (LPBE) was concentrated. Accordingly, It was justifiable to consider these sheep sera (Fig. 2), apart from serum O71 (Fig. 3), as weak positive sera with waning antibodies of old infection. Antibodies to type “O” of FMD virus, the most predominant FMD infection in Sudan, when studied in sheep sera collected in 2008 from Western Sudan and other areas lend prevalence of 9.16% (Raouf et al. 2012) similar to the one detected in this work for the 3-ABC polyproteins using the Priocheck-NS (8.47%). Evidently, the discordant Priocheck-NS positive Chekit negative could be associated with performance characteristics of these assays what helps conclusive interpretations of their results.



Strict lines are positive and negative sera by the two ELISAs (series 4 and 5 are bovine sera No. 82 and F776 respectively). Dotted lines are negative and borderline sera in Gheckit that showed positive result in Priocheck (series 1 and 2 are titrations of sheep serum No. Ao801 (two trials) and series 3 is bovine serum No. F85). N.B. true negative serum was titrated at dilutions 1/50, 1/100 and 1/150

Figure 2: Titration in Checkit ELISA: Comparison of titration curves of positive sera (the two test), negative sera (the two test) and Priocheck positive Checkit negative or borderline sera

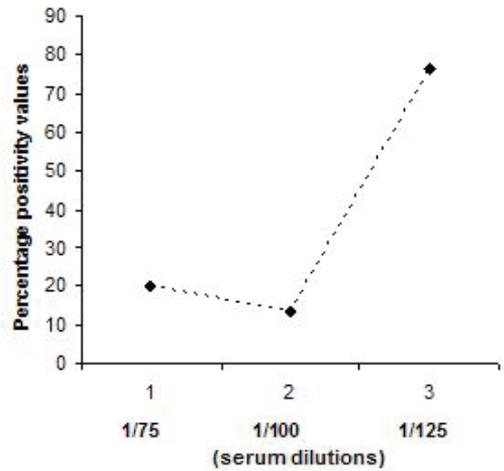
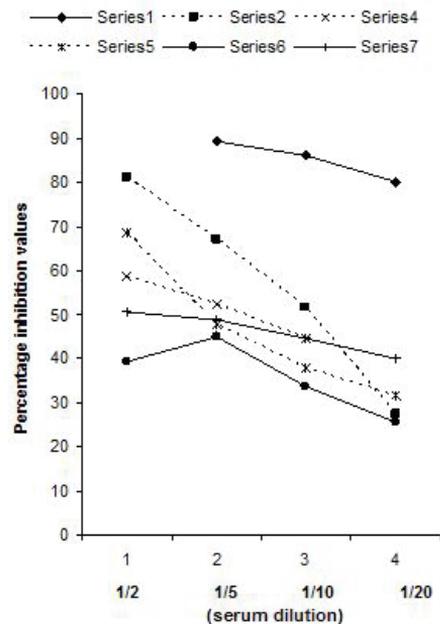


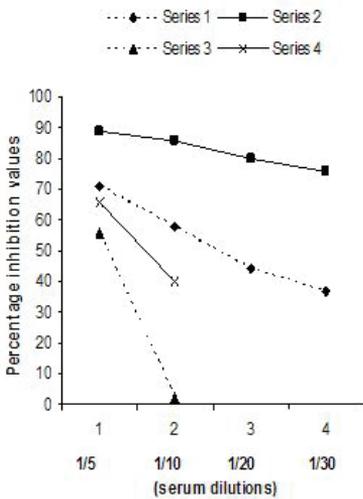
Figure 3: Titration curve of serum No. O71 in Checkit ELISA



Dotted lines are Priocheck positive Chekit negative sera (series 2, 4 and 5 are sheep sera No. O71, Ao1139 and Ao1122 respectively) Strict lines are strong positive and negative (or marginally positive) sera (series 1, 6 and 7 are sheep sera No. Ao1123, Ag 559 and Do680 respectively) The dotted line with the sharpest decline is serum No O71.

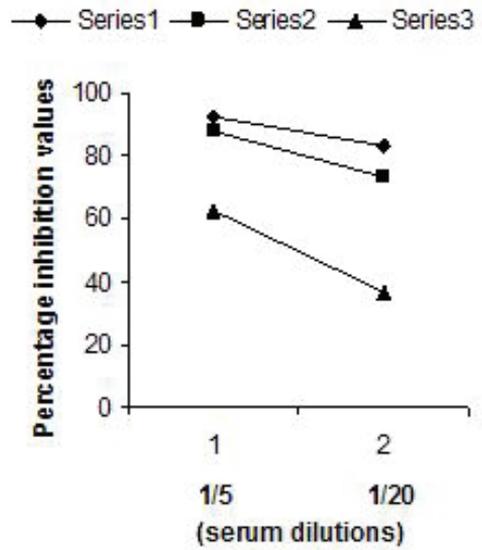
Figure 4: Titration in Priocheck-NS ELISA: Comparison between titration curves of Priocheck positive Checkit negative sera and titration curves of other sera

On the other hand the titration curve of serum O71 in Chekit ELISA (Fig. 3), unlike the rest of Priocheck-NS positive Chekit negative sera, showed positive reaction at dilution 1/125 but not at 1/75 i.e. no correlation between serum dilution and detected activity which indicated poor avidity (Growther, 2001) or non-specificity of the reaction. Characteristically, its titration curves in Priocheck-NS (Fig. 4 and 5) showed, in different trials, the sharpest decline; sharper than other titrated positive sera, what also pointed to the essentially low quantity or low avidity of the reacting component. That was particularly relevant since serum titration curves in Priocheck-NS were generally of slow decline (Fig. 6). Its titration curves bear similarity to dotted lines of titration curves in Fig (8). The latter sera changed to negative or showed lower titres in the system of blocking ELISA (LPBE type "SAT1") when higher optimum dose of the antigen was used (Raouf et al. 2007). In addition, serum O71 showed high fluctuation in positivity signal at dilutions 1/5 in the Priocheck-NS, from around 70% to above 50% or negative PI values (Fig. 4 and 5) Brocchi et al. (2006) observed that specificity of these NSP ELISAs improved on retesting.



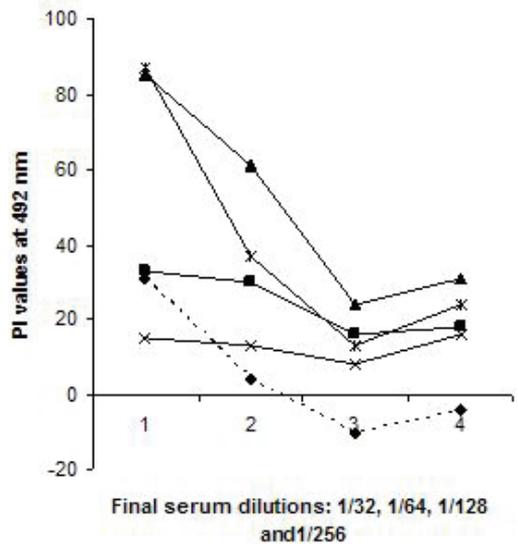
Series 1 and 3 (Dotted lines) are serum No. O71 showing sharp decline
 Series 2 and 4 (strict lines) are sera No. Ao1123 (sheep) and F85 (bovine) respectively

Figure 5: Different trials of titration of serum No. O71 in Ceditest



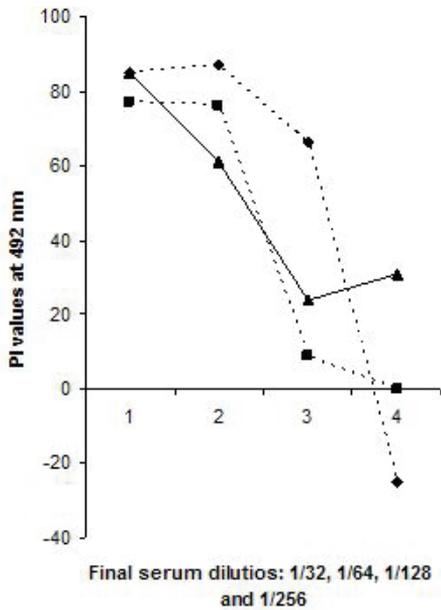
Series 1, 2 and 3 are sera No. F93 (bovine), Ao1123 and Ao1129 (sheep)

Figure 6: Slow decline of titration curves in Ceditest



Similarity to dotted titration curves in Fig (2); both groups of sera showed good titration in the system but no end point in this case or slower decline in Fig (2).
 Dotted line is the strong positive reference antiserum.

Figure 7: Titration of positive bovine sera at low dose of "SAT1" antigen in the LPBE (From Sudan journal of veterinary Research) (Raouf et al.2007)



Observe the sharp decline at one point or another in the dotted lines of titration curves.

Figure 8: Titration curves, at low antigen dose of "SAT1" in the LPBE, of sera that changed to negative or showed lower titres (dotted lines) at optimum higher antigen dose (From Sudan journal of veterinary Research) (Raouf *et al.* 2007)

When Brocchi *et al.* (2006) studied the analytical sensitivity of these two ELISAs in one serum collected 12 days post experimental infection of type "A", the highest dilution tested positive was 1/64 for the Chekit ELISA and 1/8 for the Priocheck-NS. That raises certain problems concerning the validation of results of these assays since a serum that detected positive up to dilution 1/64 in one ELISA was screened negative in the same ELISA (at dilution 1/100) whereas it screened positive in the other ELISA (1/5) though it is proved positive up to dilution 1/8 only. It is expected that sera which follow experimental infection are less avid than sera which follow natural infection because of the shorter course of infection. Accordingly, the recognition, in this work, that low affinity and large immune complexes are better detected in the system of Chekit ELISA while they are likely to decrease blocking activity in the system of Priocheck-NS would remove much of this discrepancy. It is interesting to observe that when analytical

sensitivity of these ELISAs was studied in that same work in a serum collected 13-15 days after clinical signs in an animal naturally infected with type "A", no inconsistency was detected; the serum was positive in both ELISA up to dilution 1/128.

In essence the data presented in this work strongly suggest that differences in the performance of the two ELISAs were largely due to differences in their methodology. Identification of how different methodology might affect performance is crucial for optimization of these assays and conclusive interpretations of their results. Whereas prevalence in the test group as detected by Priocheck-NS was found to be more pertinent to true prevalence, the Chekit ELISA could prove to be useful for detection of relatively recent virus circulation and, hence, control plans in enzootic areas before movement of livestock for trade or other purposes

Impact

3-ABC ELISAs are a recent and novel approach in FMD serology. They are advantageous in detection of infection of any of the seven FMD virus serotypes. Yet, in absence of a golden standard for NS protein serology and differences in performance characteristics (dSp and dSe) of these ELISAs, statistical analysis and/or confirmation of results by using two ELISAs in series (positive in both tests methods would be considered positive) or parallel (all positive results are considered) are all suggested. The presented work showed that discordant results in field sera are consistent with reported differences following experimental infection and are largely due to differences in their methodology rather than individual disparity in field sera. Accordingly, it is shown that prevalence in an enzootic area as detected by the reportedly sensitive Priocheck-NS could be more pertinent to the expected true prevalence while the Chekit ELISA could prove to be advantageous in detection of relatively recent FMD infections.

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SHORT COMMUNICATION

USE OF DATA OF AN INTERNATIONAL BOVINE SERUM EVALUATION PANEL TO DEFINE SOME PERFORMANCE CHARACTERISTICS OF CURRENT 3-ABC ELISAs

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Foot-and-mouth disease (FMD) is an important transboundary animal disease and, among viral disease, is the one with the most complex epidemiology. The seven immunological distinct serotypes of the FMD virus; "O", "A", "C", "Asia1" and the 3 South African serotypes, the wide range of animal species that contract the natural infection and rapidity and variety of methods of spread are the important factors that contribute to this fact. Tests for detection of antibodies against FMD non-structural proteins (NSP) overcome some of this complexity by detecting infection by any of the seven known serotypes (Berger *et al.* 1990; Sørensen *et al.* 1992; Bergmann *et al.* 1993). These tests are being increasingly used in endemic areas in Africa for assessment of FMD virus circulation in different animal species (Bronsvooort *et al.* 2008), before movement of animals for trade (Bronsvooort *et al.* 2004) and in country-wide sero-surveillances (Ayelet *et al.* 2012). The NCPanaftosa system, composed of an indirect enzyme-linked immunosorbent assay (Malirat *et al.* 1998) and an enzyme-linked immunoelectrotransfer blot assay (EITB) (Bergmann *et al.* 1993; Neitzert *et al.* 1991), was established by the OIE as an index screening method for verification of absence of FMD infection (OIE, 2008). Other in-house tests and commercial assays were made available for the same purposes. Frequently reported NSPELISAs (Brocchi *et al.* 2006) include the Priocheck-NS, Checkit-FMD-3ABC, SvanovirTM, IZS-Brescia in-house test and UBI[®] FMDV NS ELISA. With the exception of the UBI[®] FMDV NS ELISA, these ELISAs, including the OIE index screening method, detect antibodies to the viral NS polypeptide 3-ABC expressed as recombinant antigen in different expression system. The UBI[®] FMDV NS ELISA detects antibody to a

3B synthetic peptide. The priocheck-NS is the only blocking ELISA among the group and uses an antigen-specific monoclonal horseradish peroxidase conjugate to detect serum blocking activity, while the other five ELISAs are indirect ELISAs detect complexed antibodies and use anti-species conjugates.

The NSP serology lacks a golden standard (Goris *et al.* 2007). Not to wrongly estimate the true prevalence, statistical analysis is applied (Goris *et al.* 2007; Bronsvooort *et al.* 2008) or confirmatory test used (Kittelberger *et al.* 2008). Moreover, when these six ELISAs were concurrently evaluated in testing field sera, about 40% and 22% of results in sera originating from Zimbabwe and Israel respectively were discordant. Two levels of sensitivity were recognized; the more sensitive level was occupied by the OIE index test, Priocheck-NS and the IZS-Brescia ELISAs and a less sensitive level taken by the Svanovir, Checkit and UBI ELISAs (Brocchi *et al.* 2006). Comparing the performance of the Priocheck-NS and the Checkit ELISA in testing cattle and sheep sera from endemic areas in Africa, it was concluded that differences between these two ELISAs are principally due to differences in their methodology; whether detecting serum blocking activity (Priocheck-NS) or complexed antibodies (Checkit ELISA) (Raouf, 2015). Campos *et al.* (2008) have developed international standard sera for testing of cattle against FMD NSP antibodies. The panel of reference sera was composed of 34 cattle sera from animals with unambiguous exposed/infected status covering serotypes "O", "A" and "C". These sera were collected in South America from FMD-free areas without vaccination (FR), from regions without clinical FMD in the last 5 years but with vaccination

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(FRV), from endemic regions (E) and from cattle following FMD episodes, experimental infection or vaccination.

This communication use result data of these international standard sera (Campos *et al.* 2008) to evaluate the conclusion that differences in methodology between these ELISAs define and produce differences in their performance's characteristics (Raouf, 2015). The analysis would include all standard sera proved positive by the OIE index test ($n=23$); apart from one serum (PEI-15) collected from immunized non-challenged animal. The comparison would also involve, this instance, the OIE index test which is like the Checkit ELISA detects complexed antibodies but at a lower serum dilution of 1/20 compared to 1/100 by the Checkit ELISA. The realization of how different methodology might affect performance's characteristics is believed to be necessary for optimization of these assays and for conclusive interpretations of their result.

The cornerstone for the effect of methodology in performance characteristics' was that the formation of low affinity and large immune complexes decrease detection of serum blocking activity since they have numerous epitopes which would not be efficiently blocked while they are better detected, as they involve numerous antibodies, in systems detecting antibodies in complexes (Raouf, 2015). Low affinity or large immune complexes form when serum is of low avidity or when antigen/antibody ratio is about equal (Hugh Fudenberg *et al.* 1978). In table (1), it is evident that the only 3 standard positive sera that failed detection by the sensitive Priocheck-NS blocking ELISA were collected 10, 11 and 15-20 days following exposure. Not that merely, all 3 bled animals in this case were with no or with questionable clinical signs of FMD. Early antibodies are known to be of low avidity and mild infection, as deduced by absence of clinical signs, produce, further, less quantity and less avid antibody. It is, also, evident that none of these sera failed detection by the less sensitive Checkit ELISA; two of them were detected positive and one as suspect. The system of Chekit ELISA was described as primed for detection of early antibodies (Raouf, 2015). In

this context, it is interesting to observe that though the Checkit ELISA detects complexed antibody like the OIE index test but at lower serum concentration (1/100), yet these 3 sera showed higher percentage positivity (pp) value in Checkit than in the OIE index test (sera tested at 1/20). At the same time, though IZS-Brescia ELISA detects complexed antibody, similarly, at 1/100 of serum dilution, yet these 3 sera passed detection by this ELISA (Campos *et al.* 2008) what could further indicate particular priming of the Checkit ELISA system for detection of early antibodies such that antigen/antibody ratio in Checkit ELISA is favorable for formation of large immune complexes.

The Checkit ELISA was described as one of the more sensitive assay for detection of early infection (Brocchi *et al.* 2006) and has been envisaged to be useful for detection of recent FMD virus circulation and hence control plans in enzootic areas (Raouf, 2015). When the international standard positive cattle sera were arranged according to positivity signals in the Checkit ELISA (Table 2), it is obvious that PP values above 100% in this system were shown, exclusively, by sera collected from animals infected 2-4 weeks earlier; experimentally or naturally. Even when such recent infection sera failed to score such values, they kept scoring higher values in this system, in 3 out of 5 cases, than in the OIE index test. On the other hand, it is interesting to observe that the IZS-Brescia ELISA behaved similarly to the Checkit ELISA and produced high PP values, above 100% (Campos *et al.*, 2008), in 7 out of the 8 top listed sera in table (2). Both these ELISAs like the OIE index test detect complexed antibodies but at a high serum dilution of 1/100 instead of 1/20 in the OIE index test. The state of affair is in agreement with the presumption that at this high serum dilution the high content of low avid antibodies (recent infection) reacts preferentially to produce these high PP values.

It has been indicated that high affinity and small immune complexes are better detected in the system of the Priocheck-NS blocking ELISA (Raouf, 2015). High affinity immune complexes form when sera are of high avidity. When the panel of positive international reference sera is arranged according to

positivity signal in the Priocheck-NS (Table 3), it is evident that 4 of the 6 top values have been produced by sera collected at time expected for Ig G peak (28 days post exposure) or time expected for early antibody (Ig M) declining. Ig G peak at around 28 days and early antibody (Ig M) decline to low levels within 30-40 days post natural infection (Brown et al, 1964; Salt et al, 1993). It is evident that sera with likely avid antibodies as deduced by the date of serum collection, post exposure, or serum origin from enzootic areas showed high blocking activity in the Priocheck-NS. The Priocheck-NS was indicated as useful for routine sero-surveillance against FMD in enzootic areas in Africa (Raouf, 2015) and for screening buffalo for infection with the different types of FMDV in the continent (Bronsvort et al, 2008). It was one of the 3 sensitive ELISAs, OIE index test, IZS-Brescia in house test and the Priocheck-NS (Brocchie et al, 2006). In comparison, the OIE index performed similarly as it showed decreased averages in grouped sera in table (3) following the manner of the Priocheck-NS. It should be emphasized; it seems true that in both systems (blocking ELISA and ELISA detecting complexed antibodies) when reacting sera were used at high concentration high avid antibodies reacted more preferentially and, accordingly, the OIE index performed similar to the Priocheck-NS. Nonetheless, the difference between the two systems remains in that by decreasing the concentration of the reacting sera to 1/100 (Checkit and IZS-Brescia) the low avid sera reacted preferentially to show exceptionally distinctive high PP values above 100% that surpassed all other values within the respective test and within the OIE index.

The realization of how methodology might affect performance characteristics was used (Raouf et al, 2006 and 2007) to optimize the screening format of the liquid-phase blocking ELISA (Hamblein et al, 1986). On the other hand, concordant and discordant results of testing field sera by 3-ABC ELISAs would be more usefully interpreted. The disappearance of Checkit positive-Priocheck negative reactors in field sera from Sudan was interpreted as reflecting old FMD (older than 180 days) circulation (Raouf, 2015).

Table 1: Standard positive sera by the OIE index test which showed discordant results by the sensitive Priocheck and the less sensitive Checkit ELISAs (Campos et al, 2008)

Serum	Origin	Date of serum collection	Clinical signs	OIE index (PP)	Priocheck (PI)	Checkit (PP)	Remarks
PEI-12	Involved outbreak	in 10dpo	?	Pos (13)	Neg (34)	Pos (33)	Earliest day of serum collection in positive group
PEI-16	Involved outbreak	in 15-20dpo	?	Pos (23)	Neg (27)	Pos (39)	The 2nd of 3 animals involved in outbreak and showed questionable clinical signs. The 3rd animal scored also week positive by Priocheck ELISA
PEI-13	Experimentally vaccinated and challenged	11dpo	No	Pos (11)	Neg (27)	Susp.* (21)	
PEI-18	Experimentally infected	12dpo	Yes	Pos (31)	Pos (78)	Susp. (26)	Earliest day of serum collection in experimentally infected animals

Susp.* PP value ≥ 20 to < 30

Table 2: Arrangement of international standard sera for NSP serology (Campos et al, 2008) according to PP values in Checkit ELISA (from high to low)

Serum	Exp. Inf./date of serum collection	Natural exposure/date of serum collection	Checkit (PP)	Priocheck (PI)	OIE index (PP)	Average values
PEI-28	15dpi		245	87	70	Ch. PP: 166.88 Prio. PI: 86.13 OIE Ind PP: 80.9
PEI-26	25dpi		236	83	63	
PEI-30		15dpo	201	92	78	
PEI-32		15-20dpo	178	87	97	
PEI-25	30dpi		141	86	73	
PEI-29	15dpi		116	78	80	
PEI-22		15-20dpo	111	86	60	
PEI-33		26dpo	107	90	126	
PEI-35	37dpi		86	94	139	Ch. PP: 82 Prio. PI: 93.5 OIE Ind. PP: 102
PEI-23		240dpo	78	93	65	
PEI-34		15-20dpo	63	77	110	Ch. PP: 50 Prio. PI: 76.67 OIE Ind PP: 55.22
PEI-31		26dpo	62	86	75	
PEI-17		26dpo	53	58	30	
PEI-20	25dpi		52	75	39	
PEI-24		30dpo	49	88	55	
PEI-14		15-20dpo	49	65	11	
PEI-21		140dpo	42	77	55	
PEI-19		150dpo	41	76	53	
PEI-27		60dpo	38	88	69	

Table 3: Arrangement of international standard sera for NSP serology (Campos et al, 2008) according to PI values in Priocheck ELISA (from high to low)

Serum	Origin and clinical signs	Exp. Inf./date of serum collection	Natural exposure/date of serum collection	Priocheck (PI)	Checkit (PP)	OIE index (PP)	Averages
PEI-35	Exp/yes	37dpi		94	86	139	Pr.: 90.83
PEI-23	E/?		240dpo	93	78	65	Ch.: 93.16 OIE: 88.7
PEI-30	Outbreak/yes		15dpo	92	201	78	
PEI-33	Outbreak/yes		26dpo	90	107	126	
PEI-24	E/yes		30dpo	88	49	55	
PEI-27	E/yes		60dpo	88	38	69	
PEI-28	Exp/yes	15dpi		87	245	70	Pr.: 83.75
PEI-32	Outbreak/yes		15-20dpo	87	178	97	Ch.: 144 OIE: 78.5
PEI-25	Exp/yes	30dpi		86	141	73	
PEI-22	Outbreak/yes		15-20dpo	86	111	60	
PEI-31	Outbreak/yes		26dpo	86	62	75	
PEI-26	Exp/yes	25dpi		83	236	63	
PEI-29	Exp/yes	15dpi		78	116	80	
PEI-34	Outbreak/yes		15-20dpo	77	63	110	
PEI-21	E/?		140dpo	77	42	55	Pr.: 70.2
PEI-19	E/?		150dpo	76	41	53	Ch.: 47.4 OIE: 37.6
PEI-20	Exp/yes	25dpi		75	52	39	
PEI-14	Outbreak/?		15-20dpo	65	49	11	
PEI-17	Outbreak/yes		26dpo	58	53	30	

Non-structural proteins ELISAs are a recent approach in FMD serology. Exploring and exploiting potentialities in these tests could prove advantageous in many ways. Detection of recent FMDV circulation at a single point of time following the pattern of high PP values (above 100%) revealed by the Checkit ELISA and IZS-brescia or following appearance of positive-Checkit negative-Priocheck reactors could prove useful for control plans in enzootic areas. In many instances, in enzootic areas, direct diagnosis of FMD even in cattle, the main target species, might be problematic. Extension of these findings to small ruminants where FMD is often mild and difficult to diagnose is still more advantageous. The OIE index test and the Priocheck-NS are likely suitable tools to acquire more knowledge about the induction, duration and magnitude of immune response to 3-ABC polyproteins, given their performance characteristics' shown in Table (3).

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Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
January 2013

Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Inter-African 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. The Bulletin is the African voice on animal resources issues specific to Africa.

The Bulletin of Animal Health and Production publishes articles on original research on all aspects of animal health and production, biotechnology and socio-economic disciplines that may lead to the improvement of animal resources. Readers can expect a range of papers covering well-structured field studies, manipulative experiments, analytical and modeling studies of the animal resources industry in Africa and to better utilization of animal resources.

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