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Response of Nigerian farmers to a questionnaire on chloramphenicol application in commercial layers

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Summary

The authors assessed the occurrence of chloramphenicol (CAP) residues in commercial eggs. Of the 105 farmers surveyed for the use of CAP, 35 (33.3%) and 70 (66.7%) responded regarding their awareness or complete lack of awareness of the legislation that stipulates the use of CAP in food animals, including poultry, respectively. Only 28 (26.7%) of respondent farmers were aware that CAP was one of the drugs that is not recommended for use in food animals. Amongst the farms surveyed in the preliminary phase of this study, 24 were randomly sampled for further investigation, of which 21 (20.0%) admitted the use of CAP in forms of human and veterinary preparations, while 15 (62.5%) admitted the use of human CAP preparation only. The presence of antimicrobial residues was confirmed using specific thin-layer chromatography (STLC) in 8 out of 144 pooled egg samples (10 eggs per sample). The only positive CAP sample was identified on a farm that adopted the use of a human CAP preparation.

Keywords

Antimicrobial, Chloramphenicol, Egg, Layer, Nigeria, Residue, Risk.

Risposta dei produttori nigeriani al questionario sull'impiego del cloramfenicolo in impianti commerciali

Riassunto

Questo studio ha valutato la presenza di residui di cloramfenicolo (CAF) nelle uova presenti in commercio. Dei 105 agricoltori intervistati per l'utilizzo di cloramfenicolo, 35 pari al 33,3% e 70 pari al 66,7%, hanno risposto rispettivamente sia riguardo alla loro consapevolezza in merito, sia alla loro completa mancanza di conoscenza sulla normativa, che regolamenta l'uso del cloramfenicolo negli animali, incluso il pollame. Solo 28, pari al 26,7% degli agricoltori intervistati, erano consapevoli che il cloramfenicolo è stato uno dei farmaci non raccomandato nell'uso di alimenti per animali. Tra le aziende intervistate nella fase preliminare di questo studio, 24 sono state oggetto di una campionatura casuale per ulteriori indagini, 21 di esse 20,0% ha ammesso l'uso di cloramfenicolo sottoforma di preparati per uso umano e veterinario, mentre 15 pari al 62,5% ha ammesso l'uso di cloramfenicolo per preparati ad uso esclusivamente umano. La presenza di residui di antimicrobici è stata confermata mediante specifica cromatografia su strato sottile in 8 su un pool di 144 campioni di uova (10 uova per campione). L'unico campione positivo a cloram-fenicolo è stato individuato in una azienda che ha

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adottato l'uso di un preparato al cloramfenicolo per uso umano.

Parole chiave
Antimicrobico, Cloramfenicolo, Uovo, Impianti commerciali, Nigeria, Residuo, Rischio.

Introduction

Chloramphenicol (CAP) binds to the bacterial 50s ribosomal sub-unit to inhibit peptide bond formation and protein synthesis at the peptidyl transferase reaction (1). The drug is widely used in the treatment of human infectious diseases caused by susceptible bacteria, for instance, *Salmonella* spp. (2). Its broad spectrum of activity previously contributed to its therapeutic usefulness being extended to the management of infectious bacterial diseases of food-producing and companion animals (1). However, major toxicological health problems have been reported in genetically predisposed humans as a result of direct (therapeutic administration) and indirect (residues) consumption. Festing *et al.* (10) demonstrated the genetic predisposition of different genomes to the toxicological effects of CAP. One very important toxicological effect of CAP is the dose-independent haematological condition known as aplastic anaemia. This condition has been reported to have a poor survival rate; the only known survivor was found to develop leukaemia (2). The world health authorities have discouraged the use of this drug in food-producing animals. In view of this development, a zero tolerance level was indicated for its presence as residue in foods of animal origin destined for human consumption (16, 26).

In Nigeria, many farmers use CAP to control poultry diseases because of its claimed efficacy. This situation could result from a state of very low or absolute lack of awareness of legislation guiding the use of veterinary drugs, particularly those with zero tolerance. For this reason, there is a global recommendation for effective reporting of residues in foods destined for human consumption, especially in developing nations where poor perception of residues among farmers is being noticed (5). This situation could be worse, in which case, farmers are seen adopting human CAP preparations for veterinary use. Based on these facts, a questionnaire was structured to reflect the rate of awareness to CAP health hazards when used in poultry farming and to investigate the occurrence of CAP residues in commercial eggs in selected farms in Nigeria.

Materials and methods

Assessing the awareness of legislation guiding the use of veterinary antimicrobials

Using a competent interpreter where necessary, a set of structured questionnaires were submitted to 105 poultry farmers. The questionnaires were designed to assess the level of awareness of legislation guiding and guarding the use of veterinary antimicrobials, with particular reference to CAP, in poultry. The response of farmers in relation to the application of CAP in poultry farms were analysed statistically in association with the frequency of use of CAP using Fisher's exact test. Statistically significant differences exist at p values <0.05. A test of association was performed between the farms that adopt the use of human CAP preparation and the CAP positive-egg samples.

Study sampling

A total of 144 pooled egg samples were collected from 24 randomly selected poultry farms in Kaduna State. Each sample collected from a farm was made of 10 eggs pooled together. The sampling was performed at every interval of 14 days for a period of 3 months. Each batch of sampling was transported from the sources using crates at room temperature; these were stored at +4°C until analysis.

Sample preparation and processing

The egg yolk was separated from the egg white for general antimicrobial screening. The 8 positive antimicrobial residue egg samples (egg yolk homogenates) used in the screening were collected in Universal bottles and kept at –20°C for further specific analysis of CAP residues, using specific thin-layer chromatography (STLC).

Crude extract preparation

To every 3 g of the positive yolk homogenate sample, acetonitrile was added drop-wise while vortexing. The resulting mixture was centrifuged at 4 000 × g and the supernatant crude extract (CE) liquid was collected.

Removal of interfering substances from the crude extract

Using a modified method of Arnold and Somogyi (4), 6 ml of n-Hexane and 3 ml of distilled water were added sequentially to every 4 ml of CE. This procedure was repeated using 4 ml of n-Hexane and 2 ml of distilled water, respectively. Then, 6 ml of ethyl acetate was added to the lower aqueous phase (after a second centrifugation at 1 700 × g). The separated organic phase, resulting from the repeated centrifugations, was collected and evaporated to dryness at a temperature of 55°C.

Specific thin-layer chromatography of the dried extract

The dried extract was resuspended in 0.5 ml ethanol. The resulting mixture was spotted on thin-layer chromatography pre-coated plates and was run in a solvent system containing 90 parts of chloroform, 9 parts of methanol and 1.5 ammonium hydroxide (13). When the two-thirds solvent front was achieved, the plate was removed for drying. The dry plate was then sprayed with 1.0% tin chloride solution followed by 1.0% p-dimethylaminobenzaldehyde solution) for identification of yellow spot(s) which confirm the presence of CAP. Standard chloramphenicol powder was used as a positive control (CAPS Pharmaceuticals, SA/Pty Ltd, Johannesburg). Distilled water (Sigma-Aldrich Laborchemikalien GmbH, Seelze) was also used as a negative control.

Results

Chloramphenicol use in poultry and the occurrence of its residue in eggs

Table I shows that of the total of 105 poultry farmers interviewed to assess the use of CAP, 21 (20.0%) used CAP in the form of veterinary preparations, 15 (14.3%) administered the human CAP preparation to poultry. Only 28 (26.7%) admitted that CAP use is strongly discouraged in food-producing animals. Table II provides the results of the occurrence of CAP residues in the various sampling locations of Kaduna State. One out of the 144 pooled commercial egg samples used in the qualitative analysis presented CAP residue (0.7%). There was a statistically significant correlation between the farms which adopted the use of human CAP preparation and CAP positive egg sample.

Table I
Response of farmers regarding the use of on poultry farms

Frequency of CAP use on poultry farms	Response of farmers											Total
	Nature of CAP preparations used on farms		Staff administering drugs		Mode of administration of drugs			Poultry practice experience		Legislation guiding application of veterinary drugs		
	VPP	HP	V/PV	UTP	VP	MP	SELF	<10 yrs	≥10 yrs	Aware	Not aware	
Common	21	15	12	38	14	19	39	54	26	20	66	324
Uncommon	18	34	10	29	6	9	14	5	7	8	11	151
Never	0	12	5	11	0	1	3	10	3	0	0	45
Total	39	61	27	78	20	29	56	69	36	28	77	520

CAP chloramphenicol
VPP veterinary preparation
HP human preparation
V/PV veterinary/para-veterinary
UTP untrained personnel
VP veterinary prescription
MP manufacturer's prescription

Table II
Occurrence of chloramphenicol residues in antimicrobial-positive commercial eggs containing antimicrobials

Sampling (LGA location)	Residue positive cases		
	No. of positive eggs to antimicrobial residue	No. of farms with antimicrobial residues in eggs	No. of farms with chloramphenicol residues in eggs
Kaduna (Kaduna North)	1	1	0
Kaduna (Kaduna South)	6	4	1
Zaria Sabo Gari	3	2	0
Zaria	0	0	0
Giwa	1	1	0
Total	11	8	1

LGA local government areas

Possible effect of the use of human chloramphenicol preparation on the occurrence of its residue

Table III shows the effect of the use of human CAP preparations on the occurrence of CAP residues in eggs. All five farms that used veterinary preparations containing CAP had negative eggs for CAP. The single implicated farm with CAP residue was amongst the three farms that adopted human CAP preparation for veterinary use.

Discussion

The use of CAP in food-producing animals is globally discouraged (26). Its ban was most probably due to its likelihood of causing aplastic anaemia in susceptible consumers (20). Part of this decision was due to difficulty in determining safe residue levels (22). In Nigeria, there is poor perception of the possible effects of antimicrobial residues on human health. This has highlighted the low level of awareness of legislation that governs the application of drugs in poultry. In Tanzania, similar observations were reported with 85% of farmers, particularly small poultry holders who were not aware of the effects of antimicrobial residues on human health (18). Such poor practices among poultry farmers in developing countries have affected the frequency of drug use as observed in the current study. The entrance of veterinary drugs with no maximum residue limit (MRL), such as chloramphenicol (5), into the food chains of animal origin especially in developing nations (28) could show negative effects on the international trade market with its negative repercussions on internally generated revenues (19).

Our study showed that commercial eggs marketed for human consumption in Kaduna State, Nigeria, contain a rare case of CAP residue, despite its ban for use in food-producing animals (27). Similar reports from different parts of the world, although experimental, have demonstrated the presence and persistence of CAP residues in tissues of poultry and cattle (3, 14, 21). The seemingly rare occurrence of CAP residue as determined

Table III
Chloramphenicol preparations and related residues

Type of chloramphenicol preparation used by farmers	Detection of chloramphenicol residues	
	No. of positive farms (%)	No. of negative farms (%)
Human chloramphenicol preparation	1 (12.5)	2 (25.0)
Veterinary preparations containing chloramphenicol	0 (0.0)	5 (62.5)
Total	1 (12.5)	7 (87.5)

in our study cannot be considered insignificant. Mercer (17) and the World Health Organization (24) have reported the non-safety of CAP as residues, if present in foods destined for human consumption, irrespective of its concentration. There has not been any accurate report which demonstrates an acceptable daily intake (ADI) of CAP as residue. This further weakens efforts to determine the MRL of CAP in foods (22). A topical ophthalmologic concentration of CAP had been reported to cause bone marrow aplasia (23). Such severe and fatal outcomes may not be unconnected to certain idiosyncratic reactions. This condition had been reported to cause the death of a rancher after feeding his cattle with CAP (23).

Chances of CAP residue occurrence were currently determined to be higher, particularly in study locations or farms incriminated with high incidences of antimicrobial residues. This report depicts the true state of wrong usage of veterinary drugs amongst unqualified competitors in the poultry industries in Nigeria, which eventually leads to high chances of residue occurrences on many farms. This finding concurred with a previous report that stated 'chances of residue occurrence are higher when drugs are wrongly administered outside the recommendation of experts' (26). The risk of occurrence of CAP residue was determined to be even higher in situations where farmers adopt human CAP preparations for veterinary use as found in this study. Anàdon *et al.* (3) and Switala *et al.* (24) reported that certain pharmacokinetic variations exist when the same drug is administered to different species of animals. Switala *et al.* demonstrated the detection of CAP residues in the tissues of chickens and not blood, 12 days post treatment (24).

Wider applications of CAP to both human and animals, including food-producing animals, have bee reported (1). However, the well-established risk of irreversible bone marrow depression and the absence of safe residue levels, suddenly present the drug as unsafe for application in food animals, including poultry (6, 9). These indices gave rise to the basis which encouraged the European Union to prohibit CAP for veterinary use in 1994 (22). However, the concerns of public health authorities have been the indiscriminate use and misuse of veterinary antimicrobials as found to be common practices among livestock producers and marketers in Nigeria. This act was reported to create high-level antimicrobial drug residues (19). A lack of awareness of legislation guiding the use and marketing of veterinary drugs and the non-observance of withdrawal periods indicate that this is a serious public health threat in Nigeria (8, 12). The detection of unsafe levels of anti-microbial drug residues may present socio-economic implications. This could be a critical factor which determines the earnings of a nation on the international trade market (19).

Several methods have been reported for the detection of drug residues in raw ex-farm products (11, 15, 21). Thin layer chromatography has been affirmed to be a fast, simple, inexpensive and accurate technique that can analyse at least 10 samples at a time and be adapted for use in many laboratories (25). High-performance liquid chromatography (HPLC) is known to be very accurate but it does have limitations (7). In developing countries like Nigeria, where incorrect usage of antimicrobial drugs is commonplace, direct investigations of residues on poultry farms could be made possible by the employment of STLC.

Our study provides some preliminary information on the occurrence of CAP residues in commercial eggs on selected farms in Nigeria.

Conclusions

We demonstrated the widespread use of CAP in forms of human and veterinary preparations, which was used particularly in poultry. The use of CAP by unqualified farmers in this part of the world was observed to be due to the ineffective implementation of veterinary laws which guide and guard against incorrect veterinary usage. Such usage was seen to be a contravention of the global ban on CAP use in food-producing animals and may pose a public health risk to the susceptible consumers. Therefore, an

improvement in the existing legislation by the government, which in part empowers veterinary experts to fully execute their professional right during monitoring and surveillance of CAP usage in food animals, would make a very positive contribution to the protection of public health.

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