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Full Length Research Paper

Detection of antibiotics residues in beef in Ghanawa Slaughterhouse, Khartoum State, Sudan

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The study was planned to detect antibiotics residues present in beef in Ghanawa slaughterhouse, Khartoum State. The methodology was based on the collection of information from the owners of the cattle (n = 50), and veterinary pharmacies (n = 50) by means of questionnaire as well as the one plate test (O. P. T.) was employed for estimation of antibiotics residues in different organs of the cattle. The main results showed that most of the owners did not consult veterinarians 88% (n = 44). Furthermore, the majority of veterinarians in pharmacies 76% (n = 38) left dose restriction and administration to the owners. Using one plate test (O. P. T.) revealed that out of 300 cattle 52 (17.33%) showed positive results in one or more of their organs and there was no significant correlation level recorded for season and antibiotic residues (χ^2 = 0.720, P = 0.698, P > 0.05). Out of 300 kidneys tested, 30 (10%) showed positive result, while out of 300 livers tested, 23 (7.66%) were positive, and out of 300 muscles tested 9 (3%) were positive. The age group of 4 to 6 years scored high positive percentage (19.49%), (n = 46 out of 236 of that age group) (χ^2 = 286.260, *P* = 0.000, P < 0.01). Moreover, the cross-breed had the higher percentage of positive (20%) (n = 2 out of 10 of that breed) (χ^2 = 397.293, P = 0.000, P < 0.01). In conclusion, the findings of this work indicated that a problem of antibiotics abuse in cattle for meat production and the percentage of positive samples were considered to be high.

Key words: Antibiotic residues, cattle's meat, Sudan.

INTRODUCTION

Antibiotics are widely used in animal health practice in Sudan, as in many other countries. Occurrence of veterinary drug residues in edible animal tissues is a global problem. Residues of antibacterial drugs have been reported in slaughtered beef cattle, and veal calves (Guest and Paige, 1991; Wilson et al., 1991). Standard regulatory protocols have been established in many countries to avoid the slaughtering of animals and harboring of residues (Corrigan, 1992; Fitzpatrick, 1990; Norcross and Brown, 1991). Carcasses have also been inspected in order to eliminate residue contaminated meat from the food chain. Residues occur mostly when animals are slaughtered within the withdrawal period of the drug (Guest and Paige, 1991), and particularly when treated with an extra label dose (Krainock, 1991; Riviere, 1991). Veterinary drugs are generally use in farm animals for therapeutic and prophylactic purposes and they include a large number of different types of compounds which can be administrated in the feed or in the drinking water. In some cases, the residues may proceed from feed stuffs (Mc Evoy, 2002). But these substances may exert other effects when administrated to animals for other purposes like growth promotion. Important effects are mainly due to the presence of residual antibiotics which consist in allergic reactions or the selection of a resistant bacteria that could be transferred to human through the food chain (Butaye et al., 2001). In addition, the consumption of trace levels of antimicrobial residues

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in food from animal origin may have consequences on the indigenous human intestinal micro flora which constitutes an essential component of human physiology (Vollard and Clasener, 1994). This micro flora acts as a barrier against colonization of gastro-intestinal tract by pathogenic bacteria, (Vollard and Clasener, 1994) and has an important role for food digestion. So, the ingestion of trace level of antimicrobials in food must take into account potentially harmful effects on the human gut flora (Cerniglia and Kotarski, 1999).

In view of all these circumstances, foods of animal origin must be monitored for the presence of veterinary drug residues. Therefore, the main objective of this work was to detect antibiotics residues in cattle's meat in Ganawa slaughterhouse, Khartoum State, Sudan.

MATERIALS AND METHODS

Area of study

This study was done in Khartoum State, Sudan. Samples examined for detecting antibiotics residues were collected from Ghanawa slaughterhouse, which distributes cattle meat to most of the main meat markets and majority of the meat factories in the State.

Questionnaire survey

The questionnaire was designed in order to have basic information about the manner of using antibiotics in the Khartoum State. Information of the questionnaire was collected from fifty veterinary pharmacies: in Omdurman (30 pharmacies in Abu Zaid, Alsalam), Khartoum North (15 pharmacies in Hilat Kuku) and Khartoum (5 pharmacies in Abu Hamama). Information was also collected from fifty owners: who have cattle yards around Ghanawa slaughterhouse (35 owners), around Alsabaloga slaughterhouse (10 owners) and around Alhoda slaughterhouse (5 owners).

Collection of samples

The target samples were the tissue fluids of kidneys, livers and muscles related to the same animals and were collected by filter papers. A total of 900 samples (out of 300 cattle) were tested for antibiotics residues in different seasons.

One plate test (O. P. T.)

One plate test (O .P .T) was used as described by Korean-Dierick et al. (1995) and Nada (1996). The test organism was *Bacillus subtilis* (strain ATCC6633). The test depends on bacterial growth inhibition. Inhibition zone appears around the filter paper which contained tissue fluid of samples. The sample was considered to be positive when the inhibition zone was 2 mm and more, doubtful when it was 1 to 2 mm, and negative when it was less than 1 mm.

Sterilization

Hot air oven

This method was used for sterilization of clean glass containers, which were wrapped in paper or put in stainless steel cans, temperature was 160°C for one hour (Stainer et al., 1986).

Red heat

This method was used for sterilizing wire loops, straight wire and tissue forceps (Cruick- Shank et al., 1975).

Autoclaving

This method was used for sterilization of culture media and for materials that could not stand the dry heat. The temperature was 115 to 121°C under 10 to 15 pound pressure for 15 to 20 min (Barrow and Feltham, 1993).

Test medium

Standard nutrient agar in the form of dehydrated powder was used (Oxoid limited). The medium contained per liter; Peptone (5 g), beef extract (3 g), sodium chloride 8 g and agar No. 2 (12 g).

Test organism

B. subtilis (strain ATCC6633) was used.

Filter papers (Watt man 9)

Circle filter papers of 1 cm in diameter was sterilized by autoclaving at 121°C for 15 min in glass bottles and kept for use.

Samples handling tools

These were stainless steel forceps, blades and eppendorf tube. They were used in cutting and handling kidney, liver and muscle samples.

Preparation of samples

Sampling depends on deep incision through the target organ and a filter paper was attached for some seconds to absorb the tissue fluid, then it was transferred to abendorf tube which had a label. Abendorf tubes were kept in refrigerator at 4°C.

Nutrient agar preparation

Twenty eight grams of nutrient agar powder (Oxoid limited) was placed in 1000 ml of distilled water. The medium was autoclaved at 121°C for 15 min. The medium was cooled at 50°C. Twenty milliliters of the medium was distributed for each Petri dish and the solidified agar was kept in refrigerator at 4°C.

Preparation of standard test organism culture

B. subtilis was seeded in nutrient broth. Sporulation culture medium was perpetrated in 500 ml flat slide bottles closed loosely with screw caps for adequate aeration. Each bottle contained nutrient broth culture of *B. subtilis* and incubated at 37°C for 48 h until 90% of culture was spores.

Test procedures

One milliliters of standard organism was added to 20 ml of nutrient agar in each Petri dish. Then mixed and left for 10 min to solidify on

Table 1. Results of questionnaire survey responded by the owners (n = 50) of the cattle.

Unit	Count (%)
1. Antibiotics additions to food	
a. Yes	2 (4)
b. No	48 (96)
c. Some times	0
2. Addition of antibiotics in both drinking water and food	
a. Yes	2 (4)
b. No	48 (96)
c. Some times	0
3. Additions under supervision of veterinarian	
a. Yes	0
b. No	2 (4)
c. Some times	0
4. Consultation of veterinarian	
a. Yes	4 (8)
b. No	44 (88)
c. Some times	2 (4)
5. Presence of drugs in cattle yard administer by owner in emergencies	
a. Yes	37 (74)
b. No	10 (20)
c. Some times	3 (6)
6. Consideration of withdrawal period	
a. Yes	12 (24)
b. No	5 (10)
c. Some times	33 (66)
7. Knowledge of the owners about administration of antibiotics in purchased cattle	
a. Yes	50 (100)
b. No	0
c. Some times	0

a level surface bench. With a clean dry and sterile forceps picked up filter paper and tested. Plates were incubated at 37°C until growth is visible within 24 h. Zone inhibition was observed around the filter paper when the sample containing antibiotic was measured in millimeter by the ruler. Negative samples did not show such clear zone (Negative = less than 1 mm). Interpretation of the results was done as mentioned earlier (Nada, 1996).

Data analysis

Data was analyzed using SPSS 13.0 for windows.

RESULTS

Data on the manner of using antibiotics was obtained by means of questionnaire from the owners of the cattle (n = 50) and veterinary pharmacies (n = 50). The main results showed that addition of antibiotics in both drinking water and food was 4% (n = 2), and most of the owners 88% (n = 44) did not consult the veterinarian. Majority of veterinarians in pharmacies 76% (n = 38) left dose restriction and administration to the owners, the rest of the results are presented in Tables 1 and 2. Out of 300 cattle tested in different seasons for antibiotics residues, 52 (17.33%) cattle showed positive result in one or more organ. While, 19 (6.33%) cattle showed doubtful result and there was no effect of season concerning presence of antibiotic residues (χ^2 = 0.720, P = 0.698, P > 0.05) (Table 3). Out of 300 kidney samples tested, 30 (10%) were found to be positive and 8 (2.66%) were doubtful.

Table 2. Results of veterinary pharmacies (n = 50) questionnaire survey.

Unit	Count (%)
1. **Importance of prescription**	
a Yes	6 (12)
b. No	44 (88)
c. Some times	0
2. **Whole sale vendition for antibiotics**	
a. Yes	32 (64)
b. No	0
c. Some times	18 (36)
3. Presence of balance for estimation of weight	
a. Yes	0
b. No	50 (100)
c. Some times	0
4. Leaving dose restriction and administration to owner	
a. Yes	38 (76)
b. No	2 (4)
c. Some times	10 (20)
5. **Following up of cases by veterinarian**	
a. Yes	5 (10)
b. No	43 (86)
c. Some times	2 (4)
6. **Guiding owner to restrict dose and route of administration**	
a. Yes	45 (90)
b. No	2 (4)
c. Some times	3 (6)
7. **Guiding owners for withdrawal period**	
a. Yes	38 (76)
b. No	9 (18)
c. Some times	3 (6)
8. The most sales were broad spectrum antibiotics	
a. Yes	50 (100)
b. No	0
c. Some times	0

Table 3. Animals tested for antibiotics residues in different seasons.

Season	Tested animal	Positive	Doubtful	Negative
		Count (%)		
Summer	100	24 (24)	8 (8)	68 (68)
Autumn	100	16 (16)	4 (4)	80 (80)
Winter	100	12 (12)	7 (7)	81 (81)
Total	300	52 (17.33)	19 (6.33)	229 (74.33)

Table 4. Tested animals for antibiotic residues using different organs in different seasons.

Season	Tested animals*	Positive (Kidney- Liver- Muscle)	Doubtful (Kidney- Liver- Muscle)	Negative (Kidney- Liver- Muscle)
			Count (%)	
Summer	100	14 (14) - 11 (11) - 6 (6)	2 (2%) - 10 (10) - 5 (5)	84 (84) - 79 (79) - 89 (89)
Autumn	100	8 (8) - 8 (8) - 1 (1)	3 (3%) - 1 (1) - 3 (3)	89 (89) - 91 (91) - 96 (96)
Winter	100	8 (8) - 4 (4) - 2 (2)	3 (3%) - 7 (7) - 5 (5)	89 (89) - 89 (89) - 93 (93)
Total	300	30 (10) - 23 (7.66 - 9 (3)	8 (2.66%) - 18 (8) - 13(6)	262 (87.33) - 259 (86.33) - 278 (92.66)

*Tested animals for each organ. Cut-off point: Positive = 2 mm and more, doubtful = 1 to 2 mm, Negative = less than 1 mm.

While, out of 300 liver samples tested, 23 (7.66%) were found to be positive and 18 (8%) were doubtful. Out of 300 muscle samples tested, 9 (3%) were found to be positive and 13 (6%) were found to be doubtful (Tables 4). Cattle at age group of 4 to 6 years scored high positive percentage (19.49%) (n = 46 out of 236 of that age group), while cattle between 2 to 4 years had the lower percentage of positive (7.54%), (n = 4 out of 53 of that age group) ($\chi 2$ = 286.260, P = 0.000, P < 0.01) (Figure 1). The cross-breed cattle had higher percentage of positive (20%), (n = 2 out of 10 of that breed) ($\chi 2$ = 397.293, P = 0.000, P < 0.01) (Table 5).

DISCUSSION

The residues of antibiotics or it's metabolites in cattle's meat and other foods animal cause adverse toxic effects on consumer's health. This study of antibiotic residues in Khartoum State cattle's meat was detected hazardous for human consumption. Out of 300 cattle tested for antibiotic residues 52 (17.33%) were found to be positive, which indicated the misuse of antibiotics by veterinarian and owners as well as the absences of monitoring of antibiotics residues in slaughterhouses. Summer showed a largest number of positive animals 24 (24%), however, there was no significant association obtained for season and antibiotic residues. There was a high stress in summer resulted from high temperature's degrees and dryness of natural pastures which lead to malnutrition and diseases. In contrast, winter showed lowest number of positive animals 12 (12%) due to good temperature and natural pastures and had a good condition after autumn.

Organs tested for antibiotic residues showed that kidneys had the highest percentage of positive than other organs 30 (10%). This finding agrees with Hala (2006) (24.63%) in poultry. Most of the antibiotics excretion was done by the kidney such as animoglycosides, chloramphinicol and tetracycline. Most of the positive cases were detected in age group of 4 to 6 years (19.49%). Our results disagreed with Masztis (1984) who found (0%) result in tested bulls and calves in Canada. The disagreement could be attributed to the number of cattle tested, animal husbandry and production system in Canada. Cross-breed showed higher percentage of positive than local breeds. This is due to the fact that cross-breed is more susceptible to various diseases than local breed and this required extensive usage of antibiotics.

Questionnaire survey revealed that veterinarian did not restrict the weight of animal exactly when describing doses which lead to over-dosing or sub-dosing, and there was no following up of cases after leaving the clinic or pharmacy (86%). Furthermore, the veterinarian leave dose restriction and administration to owner (76%). Similarly, Swant et al. (2005) found the same results during his survey on antibiotic usage in dairy herds in Pennsylvania. Guiding owners for withdrawal period exist (76%). The most antibiotics selling were the broad spectrum antibiotics (100%), which indicated absence of diagnostic methods in clinics and veterinary pharmacies and they only depended on tentative diagnosis and using umbrella treatment. The same finding was reported by Ibrahim (2010) who found most of veterinary drugs selling were antibiotics and clinics and pharmacies use umbrella treatment. When cattle's yards far from center of city, the owners bought amount of antibiotics and administrated in emergencies (74%) and sometimes slaughter their animals during treatment with antibiotics (32%) or before completing withdrawal period (66%). This explained the high percentage of antibiotics residues in cattle's meat during our study. Moreover, there were no records for cattle which

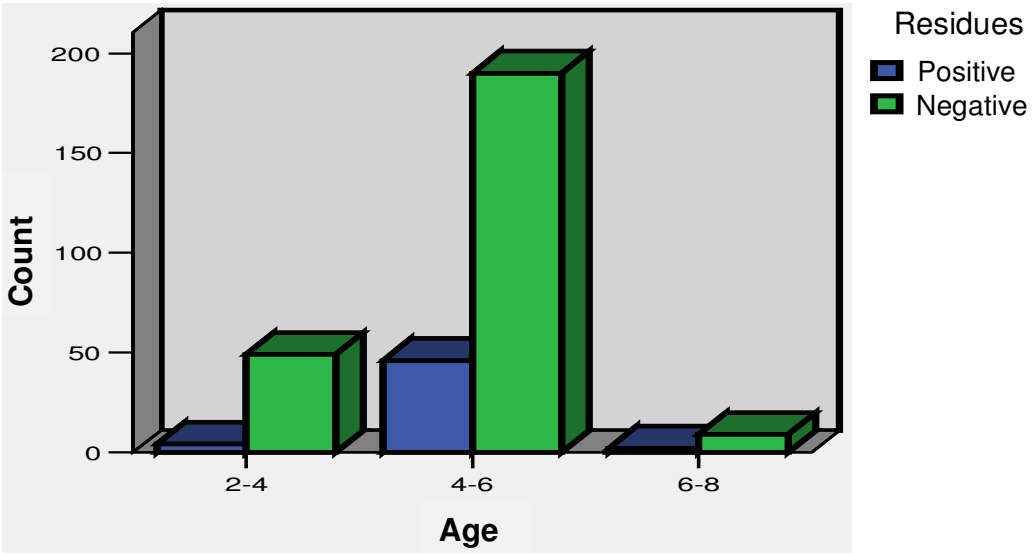

Figure 1. Association between antibiotic residues and age groups in years. Cut-off point: Positive = 2 mm and more, doubtful = 1 to 2 mm, negative = less than 1 mm, $\chi^2 = 286.260$, $P = 0.000$, $P < 0.01$.

Table 5. Comparison of antibiotics residues in different breeds.

Breed	Tested animals	Positive	Doubtful	Negative
		Count (%)		
Nialawy	147	27 (18.36)	11 (7.48)	109 (74.14)
Kinana	11	2 (18.18)	1 (9.09)	8 (72.72)
Kuror	18	2 (11.11)	0	16 (88.88)
Bugara	85	15 (17.64)	6 (7.05)	64 (75.29)
Butana	21	3 (14.28)	1 (4.76)	17 (80.95)
Qash	8	1 (12.5)	0	7 (87.50)
Cross-breed	10	2 (20)	0	8 (80)
Total	300	52 (17.33)	19 (6.33)	229 (76.33)

Cut-off point: Positive = 2 mm and more, doubtful = 1 to 2 mm, negative = less than 1 mm, $\chi^2 = 397.293$, $P = 0.000$, $P < 0.01$.

came from the markets concerned last dose administrated with antibiotics. Hence, cattle might be slaughtered during treatment period or before completing withdrawal period (100%). Our finding was previously confirmed by Hala (2006) who observed a high percentage (16.87%) of antibiotics residues in Khartoum State at Sudan.

Conclusion

Our survey indicated a problem of misusing of antibiotics in cattle for meat production and the percentage of positive samples was relatively high compared to studies in others countries. The presence of residues of antibiotics in beef as observed in the present study has a great public health concern. Human consumption of these beef may be associated with the development of antibiotic resistance in human pathogen that are difficult to treat with the commonly available antibiotics.

RECOMMENDATIONS

(1) Using antibiotics residues screening tests in meat inspection as routine.
(2) Restriction of weight exactly before describes doses.
(3) Describes doses and it's administration must be done by veterinarians.
(4) Using diagnostic methods in clinics and veterinary pharmacies before treatment.
(5) Following up cases during treatment periods.
(6) Concerning the importance of withdrawal period.
(7) Stop the whole sale vendition of antibiotics for owners.

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