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SCREENING OF ANTIMICROBIAL DRUG RESIDUES IN CAMEL IN TAMBOOL SLAUGHTERHOUSE, AL GAZIRA STATE, CENTRAL SUDAN

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Abstract:

This study was carried out to investigate the veterinary drug residues problem and the potential risk factors associated with it in Tambool area. Five hundred and seventy-four tissue samples (liver, muscle, lung and kidney) were collected at different seasons from 162 camels from Tambool slaughterhouse. One_Plate microbiological test for antibacterial drug residues was used for screening these samples. Forty-two camels (25.9 %) were positive for risk of veterinary drug residues. For the tissue samples, the results showed that there is no association with potential risk factors except in season (chi-square 80.9, P-value < 0.000) with a highly significant difference in logistic regression of Autumn (Exp (B) 45.9, P-value < 0.000) which also showed the highest percentage of positive camel samples (22.2 %). Out of 574 tissue samples, 64 showed a zone of inhibition ≥ 2 mm (35 livers, 13 muscles, 13 lungs and 3 kidneys) and 120 samples showed a zone of inhibition < 2 mm. The liver revealed the highest mean zone of inhibition (2.79 mm) from the edge of the sample in Autumn, and this is an indication of the misuse of veterinary drugs in that season, which will clearly affect the health of consumers so the animal resource ministry should pay attention and put emphasis on the development of a rapid test for antimicrobial residues and national monitoring and controlling programs must take place.

Keywords: Camel, Drug residues, One-plate test, Potential Risk Factors, Tambool slaughterhouse, Inhibition zone

Introduction

Camels are multi-purpose animals used for riding, drought work and racing. Rearing camels for most Sudanese owners is considered a good source of wealth and existing way of life. In some tribes, urine and milk are used for medical purposes (Falah 2004, Ishag *et al.*, 2011, Shuiep *et al.*, 2014 and Osman *et al.*, 2015). Fay *et al.* (2011) reported that camel meat is rich in protein and with low cholesterol and fat, so camel owners prefer to consume camel meat rather than the other types of meat (beef and mutton).

Veterinary drugs have a basic role in animal husbandry and management as they are used as: prophylaxis, metaphylaxis, treatment and some antibacterial agents are used as growth promoters for muscle development and increase water retention to increase the animal weight for more economic benefits (Kirbis 2007, Reig and Toldrá 2007 ; Page and Gautier 2012). Veterinary drugs used in Sudan are considered a potential hazard to the consumers due to improper usage and the failure to abide by the withdrawal period because the owners usually, do not consult veterinarians (Wahab *et al.*, 2011), residues of these veterinary drugs may remain in tissues such as liver, kidney and muscle ...etc and may pose a real public health concern, not only due to the presence of antimicrobial residues in edible tissues which can cause allergic reaction in hypersensitive individuals or disruption of intestinal normal flora,

but also due to emergence and transfer of bacterial resistance or multi-drug resistance, carcinogenicity, mutagenicity, teratogenicity, and ultimately destroy the economy (Kirbis 2007, Beyene 2016 ; Wang *et al.,* 2021).

In general to regulate the usage of veterinary drugs in the field, the maximum residue limits (MRLs) and withdrawal periods should be investigated to determine the concentration of the residues present in the animal and animal products (kidney, liver, lung and muscle). Ali *et al.,* (1996) reported that, the larger animal species have a lower metabolic rate and in certain cases camels may metabolize some drugs differently due to different anatomical structures, physiological and biochemical processes. This result was consistent with what El Emam (2011) mentioned in his study that the withdrawal period in camel was found to be longer for some drugs (ampicillin, oxytetracycline and sulphadimnidine) as compared to cow and other animals.

Ghada *et al.* (2018) study indicated a two-week withdrawal period for tetracycline, but more investigation of the withdrawal period of antimicrobial drugs in camel has not as yet been studded, therefore these evaluations ensured that food derived from treated animals is safe for human consumption with recommendation to estimate withdrawal period for every drug in camel and not extrapolated of doses from other animals (El Emam 2011), also it is recommended that meat must be well cooked to reduce exposure to antimicrobial residues (El-Ghareeb *et al.,* 2019).

To the best of our knowledge, there is no previous study concerning antimicrobial residues and the potential risk factors associated with camel in Sudan regarding public health, so this study was carried out in the biggest area of camel meat consumers to investigate the antibiotic drug residues problem and the potential risk factors associated with it.

Material and methods

1. **Study population:**

The study was conducted on 156 camels brought to the slaughter-house; also there are six livers samples of six different camels from butcheries without any animal information. The camels were categorized according to age, gender, breed, body condition and type of production during the anti-mortum stage and marked on the head or the top of hip joint (owners used to take their carcass without doing skinning process in the slaughter-house, because they had an idea that keeping the skin will save the meat from drying).

2. **Study area:**

This study was conducted in Tambool in the Butana area-mid-Eastern Sudan, over three seasons (winter, summer and autumn) in 2018. The samples were mainly from Tambool slaughter-house.

3. **Study design:**

A cross-sectional study among three seasons of 2018 was used.

4. **Sampling:**

From each camel slaughtered four samples of 150 mg each were collected (lung, liver, kidney and muscle) using zip lock bag for each organ separately and put all at one bag labelled by the camel code symbolized to all camel information (type of organ and date). Samples were kept in an icebox and transported directly to the laboratory of Tambool Camel Research Center and were kept until sent to bacteriology department at the Central Veterinary Research Laboratory _Soba, Khartoum State.

5. Laboratory method:

Microbiological inhibition test was used, (Koenen-Dierick *et al*, (1995)) modified to One_Plate Muller-Hinton agar for better diffusion of antibiotics in sample if there are residues.

5.1. Preparation of inoculants:

The microbes *B.Subtilis* were obtained from bacteriology department. The primary culture was refreshed by sub-culturing into nutrient agar. A single well-isolated colony of *B.Subtilis* was inoculated in 5 ml normal saline and turbidity was adjusted to 0.5 McFarland opacity standards Shahid *et al.*, (2007).

5.2. Preparation of the plate:

Muller-Hinton media prepared as an instruction mentioned by the manufacturer, then auto cleaved at 121 C^o for 15 min. cooled and poured into Petri dishes and allowed to solidify, after that inoculated at 37^{o}C over-night. The *B.Subtilis* was inoculated on the media by using a sterile swab in one way direction.

5.3. Preparation of the sample:

One piece of tissue (10*2 mm) was put over the media and incubated at 37^{o}C over-night for the production of zone of inhibition. Also, all four organs for each camel were put over one plate; note that animal with inhibition zone at least around one organ was considered positive for antimicrobial residues.

5.4. Reading results:

Detection of transparent zone from the edge of the tissue sample and the outer limit of the inhibition zone was considered as a positive result for the presence of inhibitory factors, if it gave an inhibition zone equal or superior to 2 mm in width. Gondová *et al.*, (2014)

6. Statistical analysis:

Exploratory data analysis was done using SPSS statistical software (Statistical Package for the Social Sciences) version 20. Univariate analysis tables that used chi-square test, describe the number of camels, percentage, degree of freedoms and chi-square p-value (≤ 0.05) which explains if there is a significant difference between veterinary drug residues and the potential risk factors by using confidence interval 95% among the slaughtered camels. Also logistic Regression was used for significant factors (p-value of ≤ 0.05).

Results

The results showed that forty-two of the camels examined 25.9 % (Autumn 22.2 %, Winter 2.5 % and 1.2 % in Summer) were positive for veterinary drug residues as illustrated in Figure (1).The camel is considered positive if only the zone of inhibition $\geq$ 2 mm occurs at least around one organ Table (1).

From a total of 574 tissue samples, 64 showed a zone of inhibition $\geq$ 2 mm (35 livers, 13 muscles, 13 lungs and 3 kidneys) and 120 samples showed zone of inhibition < 2 mm, most of them in the kidney 40.1% Table (1).

Figure 2 illustrated that the rainy season (Autumn) showed the largest diameter mean of ZI around all organs compared to winter and summer 2.3 mm for liver, 1.3 mm for lung 1.0 mm for muscles and 0.4 mm for kidney.

Results showed that out of 162 camels examined, 146 were collected from slaughterhouse, 10 camels without animal information and 6 samples (6 liver samples from six camels) from butcheries as described in Table (2).

The chi-square test results showed that there is no significant difference in all potential risk factors except for seasons (Table 3); the logistic regression analysis in Table (4) explained that veterinary drugs were used extensively in rainy season. Although there is no significant

difference in the breed the results showed that the positive rate was higher among the camels originated from Darfur, but it clearly demonstrated the excessive use of veterinary drugs due to closed farming system for producing meat.

The majority of positive camels had a bad body condition by (13.7%) without showing any signs of illness and all of them were in closed system (Table 3).

Discussion

Microbiological methods are used in many different studies related to antibacterial residues and in our present qualitative screening study we focus on antibacterial drug residues in camel tissues and risk factors. It's worth mentioning that this study is considered the first to be conducted in camels in Sudan, so our finding was compared with researches concerning other species.

A high percentage of positive samples was found in the liver (22.2 % out of 25.9 %) indicating a risk for consumers health, especially those who consumed raw liver as a habit this result is higher than that obtained by Wahab *et al.,* (2011) in cattle liver (7.66 % out of 17.33%)

On the other hand, the percentage of veterinary drug residues occurrence in liver samples of slaughtered camels are much lower when compared with findings reported in sheep in Sudan by Fangama *et al.,* (2019); they found that veterinary drug residues in liver samples were detected at percentages of 25 % compared to what has been reported in this study 22.2%

In summer the slaughtered camel showed no problem with withdrawal period of drugs, and the percentage of positive cases was 1.2 %, this result was not in line with the result of Hind *et al.,* (2014) and Tasneem *et al.,* (2021), who reported that the use of veterinary drugs in poultry industry was much higher in the summer season by 27 %, 35.8 % respectively.

Kaneene and Miller (1997) mentioned that the age and use of animals are the main factors associated with residues, and our study is in agreement with the previous statement that animals used for meat production have more residues than those who driven to the slaughter-house. The variation in the body condition among the animal in our study was statistically in significant difference, however, the occurrence of drug residues in bad body condition animals (20 of 79 camels) was higher by (13.7) %, than those with good body conditions might probably indicate that the health status also has an effect on pharmacokinetics and withdrawal period of the veterinary drugs. The study also showed that natural grazing is much better than closed intensive breeding system, as residual rate was higher among the closed system, which is estimated at 19.9% of the total percentage, which is 25.9 %.

In Egypt Morshdy *et al.,* (2015) results showed that the veterinary drug residues are not such a problem for camel meat consumers which are obviously opposite to our results, and he attributes that to the high immunity profile in camel compared with other animals and rapid metabolism for recently administered antibiotics in the camel.

In this study antimicrobial screening method was used to detect the presence of antibacterial residues, a confirmatory method is required to determine which antibiotic is wildly used among seasons. Another method of choice to determine concentrations of veterinary drugs is a thin-layer chromatographic (TLC) method which was validated by LC-MS in Al-Amri *et al.,* (2021) study; also There are other techniques (Gas Chromatography, high-performance liquid chromatography, ELISA and CHARM II) provided results that were reliable and precise for the detection of chemical residues in meat and meat products Al-Amri *et al.,* (2021). Also in our work, we successfully determined the potential risk factors that might be associated with drug residues as a research hypothesis, but these results were limited to Tambool area,

however, it cannot be generalized to all camels in Sudan. However veterinary drug residues are a big problem that deserved further investigation.

A veterinary drug residue is a serious problem for consumers of camel meat and might be a source of antibiotic resistance in the future, so the animal resource ministry should pay attention and put emphasis on the development of a rapid test for antimicrobial residues and national monitoring and controlling programs of antimicrobial residues in food animal origin must be done. In addition, to raise awareness of veterinary drug residues workshops for owners and consumers must take place.

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Tables and Figures

Table 1. Zone of Inhibition length around the edges of the camels' tissues using One_Plate microbiological test

Tissues	Total (574)	ZI (%)		
		No IZ	**< 2 mm**	≥ 2 mm
Liver	156	106 (67.9)	15 (9.6)	35 (22.4)
Lung	146	102 (69.9)	31 (21.2)	13 (8.9)
Kidney	117	67 (57.3)	47 (40.1)	3 (2.6)
Muscle	155	115 (74.2)	27 (17.4)	13 (8.4)

ZI : Zone of Inhibition

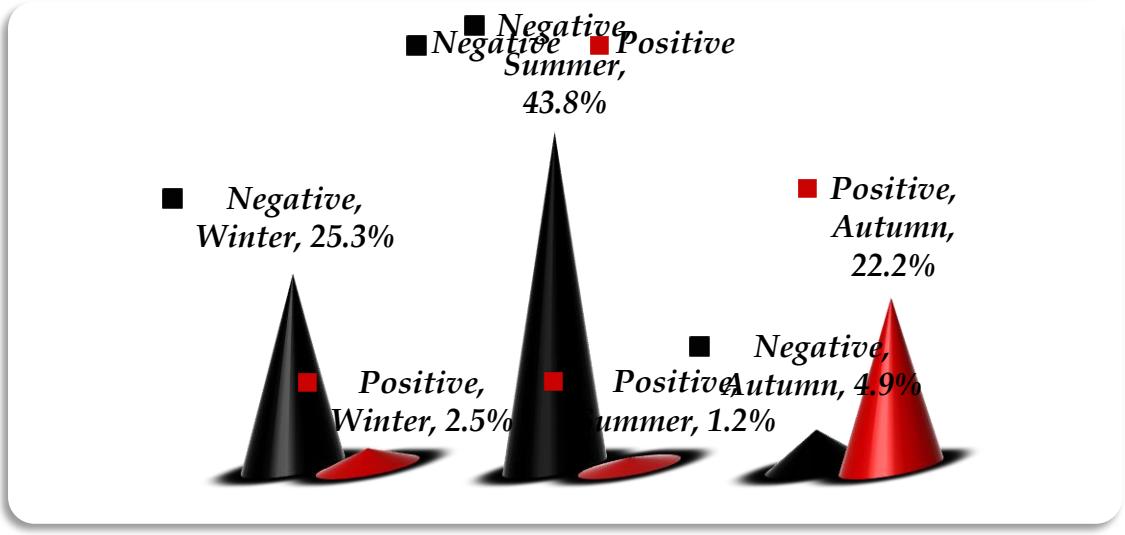

Figure 1. Screening of veterinary drug residues of camel among the three seasons of 2018 in Tambool

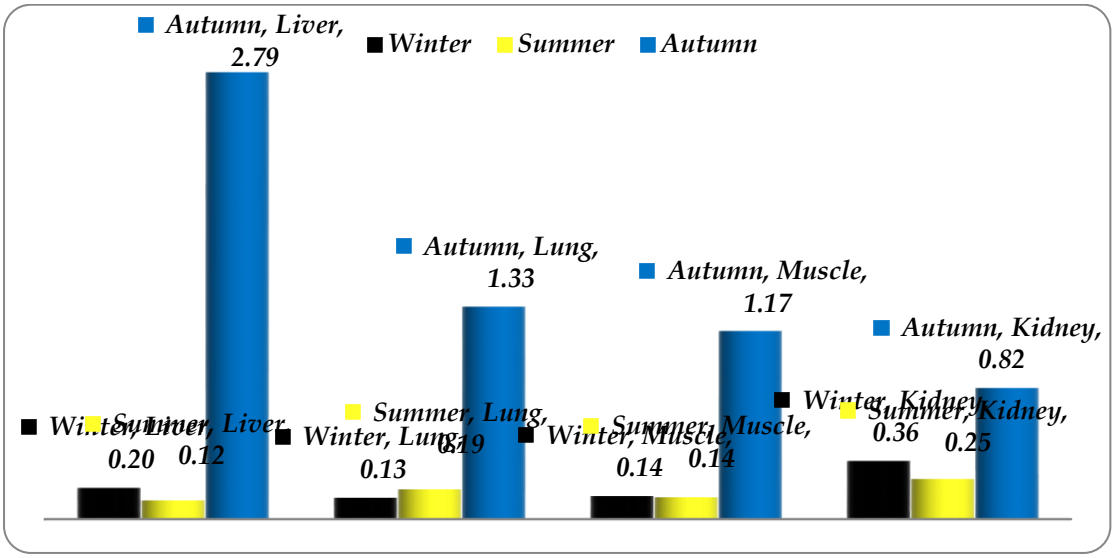

Figure 2. Mean of ZI around tested organs among seasons in Tambool

Table 2. Distribution of the sample of camels and camel products collected from Tambool

Samples origin	Positive (%)	Frequency	Valid Percent	Cumulative Percent
Tambool Butcheries	6 (3.7)	6	3.7	3.7
Tambool slaughter house	29 (17.9)	146	90.1	94.4
Samples without animal information	7 (4.3)	10	6.2	100.0
Total	42 (25.9)	162	100.0	

Table 3. Summary of veterinary drug residues of the camel and potential risk factors in 146 camels slaughtered in Tambool slaughterhouse using chi-square test:

	Frequency (%)	Positive (%)	Df	Chi-square	P-value
Season			**2**	**80.9**	**0.000****
Winter	45 (30.8)	4 (2.7)			
Summer	72 (49.3)	2 (1.4)			
Autumn	29 (19.9)	23 (15.8)			
Breed			**3**	**4.98**	**0.418**
Buttana	44 (30.1)	9 (6.2)			
Darfur	92 (63)	17 (11.6)			
Kenana	7 (4.8)	2 (1.4)			
Others	3 (2.1)	1 (0.7)			
Age			**2**	**0.35**	**0.837**
<7	68 (46.6)	13 (8.9)			
7-14	49 (33.6)	11 (7.5)			
>14	29 (19.9)	5 (3.4)			
Gender			**1**	**0.019**	**0.889**

Male	19 (13)	4 (2.7)			
Female	127 (87)	25 (17.1)			
Body condition			**1**	**3.21**	**0.073**
Good	67 (45.9)	9 (6.2)			
Bad	79 (54.1)	20 (13.7)			
Production			**1**	**1.57**	**0.282**
Meat	144 (98.6)	28 (19.2)			
Milk	2 (1.4)	1(0.7)			
Feeding system			**1**	**0.75**	**0.384**
Concentrate	143 (97.9)	29 (19.9)			
Natural grazing	3 (2.1)	0			

Table 4. Logistic Regression for Season factor:

Seasons	Sig	Exp(B)	95% C.I. for EXP(B)	
			Lower	Upper
Winter	-	Ref.		
Summer	0.167	0.29	0.05	1.65
Autumn	0.000**	39.3	10.0	153.7

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