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PERFORMING THE SWAB TEST ON PREMISES (STOP) FOR DETECTING ANTIBIOTIC RESIDUES IN CALVES

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SUMMARY

Wide use of antibiotics in veterinary practice evoked the development of rapid techniques for detection of their residues in tissues of food animals.

A preliminary experiment for detection of antibiotic residues in spiked samples was done beside a trial to investigate the distribution of antibiotic in different tissues (muscle, heart, kidney, liver and serum) of animals after intravenous injection of antibiotic (6 sheep less than one year old were used).

Applying the Swab Test On Premises (STOP) and the Life Animals Swab Test (LAST) on kidney and urine of 50 calves salughtered at Cairo absttoir reveals the presence of antibiotic residues in 10% of examined calves.

NTRODUCTION

Antibiotics in veterinary medicine are used for reatment of infectious diseases, prophylaxis and s feed additives (FAO/WHO., 1980).

Utilization of antibiotics in feed to promote rowth started in the 1940s and has been ontinued with very little control until recently Hubbert and Hagstad, 1991).

Residues of antibiotics are detected by many tecniques among which, High Performance Liquid Chromatography (Aszalos, 1985 and Moats, 1990); Radioimmunoassay (Norcross and Brown, 1991) and Bacilue subtilis BGA test (Nouws et al., 1979).

In the last few years, the Food Safety and Inspection Service (USDA) has developed a series of rapid test for detecting the illegel residue.. They are inexpensive and faster than the forecited laboratory techniques. Moreover, it could be conducted on- the spot in slaughtering or processing plants (FSIS, 1991 a). The rapid tests include:-

Swab test on primises (STOP) f or detection of antibiotic residues in livestock kidney tissues; Life animal swab test (LAST) for detection of antibiotic residue in urine of living animals; Sulfe on site (SOS) for detection of sulfamethazine in swine urine and Cattle urine test (CUF) for detection of chloramphenicol in cattle.

The current investigation was planned to screen antibiotic residue in veal calves using the new rapid tests. It was preceded by:-

1- Evaluation of the Stop test on premises (STOP), High performance liquid Chromatography (HPLC) and the usual antibiotic sensitivity test.

2- Following the distribution of the antibiotic residues in animals to select the suitable tisue for applying the rapid test.

MATERIAL AND METHODS

A) Experiments and materials used:-

1- Recovery experiment:

Samples from lean meat and kidney were spiked with 0.1,0.5, 1 and 10 ppm of Oxytetracyline (OTC), obtained from Sigma Chem.Co. ST. Leuis M O 63118, and examined for detection of antibiotic by HPLC, B.subtilis BGA method and STOP. Results were recorderd in Table (1).

2-Distribution and degradation of oxytetracycline residue in animal tissues:

Six sheep, less than one year old, weight 25-30Kg were received single i.v dose of Liquamycin LA-200 Pfizer "Oxytetracycline injection antibiotic" at a rate of 50 ul/kg.

The first sheep was dispatched 4 hours after injection, followed by slaughtering of one sheep daily and after one and two weeks.

Residues were detected by HPLC in serum, muscle, heart, liver and kidney. Results were recovered in Table (2).

3- Application of STOP and LAST as a rapid screening test for antibiotic residues in calves:

Fifty veal calves (male buffaloe, about 6 weeks old and 60 kg body weight) were examined before slaughter by LAST for screening antibiotic residues in their urine. After slaughtering, their kidneys were examined by STOP. Results are recorded in Table (3).

B) Methods

Animal residues were detected by the following methods:

1.High performance liquid chromatography (HPLC) (Ibrahim & Moats, 1994):

The apparatus used was a varian (Sugarland TX) 9010 pump, a Waters (Milford, MA-WISP 712 autosampler with a 2000 ul loop, a Polymer laboratories (Amherst, MA) PLRP-S column (5um Particle size, 4.6x 150mm, 100A pore size, with matching guard column), and a waters 990 diode array detector.

2. Test for antibiotic residue:

The B.subbtils BGA test recommended by Nouws et al., (1979) was used.

3. STOP (FSIS, 1991a):

Is a microbiological screening test for antibiotic residues in animal tissues developed by the FSIS, USDA and applied in the slaughterhouses since-1979

In this test, cotton swabs saturated with tissue fluid are placed on a culture plate seeded with B.subtilis and incubated overnight at 30°C. Presence of zones of inhibition around the swabs is presumptive evidence that the carcass tissues contain antibiotic residues. Neomycin disc (N5) was added to the plates as control to show the test is working normally.

Sterile cotton swabs, STOP agar plates, B. subtilis spore suspension and Neomycin antibiotic sensitivity discs (N5) were obtained from Food Safety and Inspection Service, USDA, Minneapolis, MN 55403.

4. **LAST** (FSIS, 1983);

is a modification of the STOP, used for detection of antibiotic, residues in urine of animals.

The cotton swabs are dipped in urine and placed on the seeded STOP agar plate

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RESULTS

Table (1): Detection of added antibiotic by different methods

Tissue	OTC added ppm/g	Method of detection			
		* HPLC			
		OTC detected	Recovery (%)	B. subtilis BGA	STOP
Muscle	0.1	0.0949	94.9	-	-
	0.5	0.4810	96.2	-	-
	1.0	0.9738	97.4	+	+
	10.0	9.8660	98.7	+	+
Kidney	0.1	0.0950	95.0	-	-
	0.5	0.4800	96.0	-	-
	1.0	0.9560	95.0	+	+
	10.0	9.9800	99.8	+	+

* Average recovery by HPLC 96.7 % for both tissue

Table (2): OTC-residues in animal tissues after intravenous dosing

Time	Serum	Muscle	Heart	Liver	Kidney
4 (hrs)	3.0817	2.4166	3.3016	8.1566	22.3866
24 (hrs)	0.1508	0.1908	0.2621	0.3317	1.1423
48(hrs)	0.1214	0.1717	0.2294	0.3164	0.9692
72 (hrs)	0.0717	0.0474	0.0602	0.1198	0.3362
1 week	0	0	0	0.0553	0.0478
2 weeks	0	0	0	0.0065	0.0323

Table (3): Incidence of antibiotic residues in calves

Test	No. of examined samples	+ve samples		-ve samples	
		No.	%	No.	%
STOP	50	5	10	45	90
LAST	50	5	10	45	90
STOP & LAST		5	10	45	90

DISCUSSION

Antibiotics were first discovered in the 1940s as the by products of fungal and bacterial growth (Libby, 1975). In USA, more than 40% than of produced antibacterial agents are for animals (Hubbert & Hagstad, 1991). Nearly 100% of poultry and 60% of cattle received antibacterial feed supplements.

Measureable violative antibiotic residues may occur with any mode of administration (Moats et al., 1985). With animals normally treated by mass treatment there is seldom a residue problem, while there is a problem with animals treated on an individual basis for specific illness (Libby, 1975), especially if there is non-withdrawal of antibiotic therapy before slaughter (Adetosoye , 1986).

Antibiotic residues in animals tissues are of significance for both animal and human, Subtherapeutic doses of antibiotic for prophylaxis or as feed additive may favour the selection and development of resistant and R-plasmid bearing bacteria which may produce human infection (Hubbert & Hagsted, 1991). Ingestion of meat and meat products containing residues may increase the prevalence of resistant bacteria in human. Moreover, the possibility of allergic reaction in some sensitive persons to antibiotics may exist (Andriessen, 1987).

In Germany, all animals which are slaughtered in emergency are to be examined for antibioics. In addition, about 1% of the slaughter animals selected at random are to be tested in the same way (FAO/WHO, 1980). While in Egypt, testing carcasses for antibiotics resides is only done for carcasses examined bacteriologically.

In the current investigation, a perliminary recovery experiment was carried out to detect Oxytetracycline added in different concentration to kidney and muscle tissue by different techniques. Obtained results (Table 1) reveal a high recovery by HPLC reaching about 96.7% even at low concentration.

On the other hand, there was a complete coordination between the usual anibiotic sensitivity test (B.subbtilis BGA) which is the main official method used in meat inspection to detect residues and the new field STOP rapid technique, but both were able to detect Oxytetracycline residue at a conentration over Ippm which is 10 times the permissible residue limit in edible cattle tissue (FSIS, 1991b).

The risk of antibiotic residues in food animals is not confined to the consumption of their flesh, but it extends also to involve their edible offals especially kidney, liver and heart which retain higher residues and persist longer than in muscle. Recorded results in Table (2) emphasized this statement, confirming also the importance of allowing treated animals a suitable withdrawal time to have a safe meat.

The highest recovery of Oxytetracycline residues was in kidney (22.38 ppm) which represent about 3 times the detected residues in liver (8.15 ppm) and 10 times that in muscles (2.41ppm).

Bob calves represent an acute problem because they are slaughtered before many administered drugs can deplets to safe concentration (Norcross & Brown, 1991). Moreover, in Egypt, they are slaughtered on arrival without having a sufficient preslaughter rest. In the current investigation STOP as rapid techniques for screening of antibiotic residues in kidney of food animals were applied. Residues were detected in 10% of examined calves (Table 3).

Similar recovery rate was detected by Forschner (1962). Higher figures (35.5%) and (20.6%) were detected in kidneys of calves by Beck & Mantel (1972) and Draheim (1971) respectively.

Contrarily, Norcross & Brown (1991) detected antibiotic residues in only 3.2% of examined calves kidneys.

Unfortunately, all examined calves in this study passed the routine abattoir post-mortem inspection and released as fit for human consumption.

The threat of entering such meat the food chain is that all cooking procedures and cold storage could not destroy completely such residues (Andriessen, 1987; Moats, 1988, and Ibrahim & MOATS 1994).

Testing of live animals for residues is a new concept. LAST technique was carried out in this investigation on calves urine . Antibiotic residues was detected in the same animals (Table 3). This finding shows the importance of testing animals, which have been treated with antibiotic and are ready for slaughter, for residues in farm or slaughterhouse quarantine with detaining of positive animals for suitable withdrawl periods to ensure the production of safe meat.

Finally, and with the freeding of use of antibiotic, the producers must accept the responsibility to use antibiotics wisely and correctly to ensure that no innocent party is hurt by their use.

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REFERENCES:

Adetrosoye, A.I. (1986): Carcass contamination and antibiotic residue in slaughter houses in Ibadan, Nigeria. Bull. Anim. Hlth. Prod. Afr., 34, 99-101.

Andriessen, F.H. (1987): Meat inspection and veterinary public health in Australia, Righy, Publ., Sydney, Auckland & London.

Aszalos, A. (1985): Fast determination of tetracycline antibiotics in different media by High performance liquid chromatography. Chromatographia, 20,5, 313-321.

Beck, G. and Mantel, T. (1972): The biological intibition test- a means of clarifying the residue problem in meat. Fleischwirtschaft, 52,12, 1605-1609.

Draheim, R. (1971): Detection of antibiotic residues in bacteriological meat inspection . Monatsheft fur Veterinarmedizin, 26,3, 511-519.

FAO/WHO (1980): Hygiene and control of meat and food of animal originSeminar 11-18 Dec., 1980, Nicosia, Cyprus.

Forschner, E. (1962): Hemmstoffe in Organen von Schlachttieren. Arch. fur Lebensmittelhygiene, 13, 242.

FSIS (1983): How to perform the live animal swab test for antibiotic residues. USDA, Agriculture Handbook 601.

FSIS (1991a); STOP for detection of antibiotic residues in livestock kidney tissue, USDA. FSIS.

FSIS (1991b): Compound evaluation and analytical capability National residue program plan. USDA, USA.

Hubbert, W and Hagstad, H. (1991): Food safety and quality assurance, IOWA State Univ. Press, Ames.

Ibrahim, A. and Moats, W. (1994): Effect of cooking proceedues on Oxytetracyline residues in lamb muscle. J. Agric Food. Chem., 42, 11, 2561-2563.

Libby, J. (1975): Meat hygiene, 4th Ed., LEA & Febier, Philadelphia.

Moats, W. (1990): Liquid chromatogaraphic approaches to antibiotic residue analysis. J.A.O.A.C., 73,3 343-346.

Moats, W.; Harris, E. and Steele, N. (1985): Drug residues in animal tissues. J.A.O.A.C., 68, 3, 413-416.

Norcross, M. and Brown, J. (1991): Food safety and inspection service, initiatives for tissue residue reduction in meat and poultry , JAVMA, 1985,5, 819-824.

Nouws, J.; Van Schthorst, M. and Ziv.G. (1979): A critical evaluation of several microbiological test methods for residues of antimicrobial drugs in ruminants. Arch, fur Lebensmittelhygiene, 30,1, 4-8.