

V. DISCUSSION AND CONCLUSION

5.1. Discussion

5.1.1. Proportion of tetracycline antibiotic residues

A proportion of 5.52% samples indicated positive to only tetracyclines antibiotic residue implies that there might be a much higher proportion of residues of antibiotics. In general, the frequency of utilization of tetracycline group accounts for 7.95% total utilization of antibiotics in pig and chicken production (Thuận *et al.*, 2003). The injudicious use of antibiotics (dosage regimen violation, no observance of appropriate withdrawal time, etc.) is commonly seen at animal farms (Boisseau, 2002; Thuận *et al.*, 2003). In addition, according to Boisseau (2002), a combination of antibiotics is quite popular in the country with 66.3% VMPs containing more than one antimicrobial.

This proportion is much higher one. It was reported that in Western countries such as the UK (Gracey *et al.*, 1999) 44 out of over 12,300 samples collected from pigs contain chlortetracycline. However, this proportion is much lower than one found in Ireland with 42 % and 12 % pork samples detected positive to chlortetracycline for the period 1996/1997 and 1997/1998, respectively (TEAGASC, 2001).

To date, no similar study on the prevalence of tetracycline residues in pork in the country has been reported. However, a study in the southern province of Binh Duong reports proportion of tetracycline residues in incurred (suspicious) pork samples with oxytetracycline detected in 7 (14.6%) and chlortetracycline 6 (12.5%) out of 48 samples (Thuận *et al.*, 2003).

A significant difference between the two selected areas in antibiotic residue proportion was found in this study. An odd ratio (OR) calculated as 4.03 showed a four-time higher likelihood in getting product contaminated with the antibiotic. A

possible explanation for this might be the socio-economic conditions of the two areas. The living standard is usually lower in the suburban area than the urban area. In the suburban, consumers' awareness of food hygiene and safety is generally poorer and a less strict inspection of slaughter and trade of animals are assumed. It might be possible that the shop owners tend to buy cheaper products of live animals. The cheap products might be generally originated from sick or even dead animals that had very likely undergone an antibiotic treatment just before slaughter.

Although there is no statistically significant difference ($\alpha = 0.05$) in antibiotic residue proportion in relation to residence of owner, the P-value of 0.08 that is very close to the significant level ($P \leq 0.05$) may be explained by a fact that most meat shops located in the urban area are owned by people from this area, and similarly for those in suburban area.

5.1.2. Concentration of tetracycline compounds in pork

All the six samples, in which quantities of tetracycline compounds were confirmed and quantified, were collected from the suburban markets. This may support the conclusion above that there is a higher risk of getting antibiotic residues in pork in the suburban areas than getting them in the urban area.

Three tetracycline compounds were identified in samples. This finding may possibly indicate that these three substances were being still used widely in animal production and that the withdrawal time for these antibiotics was not properly observed.

There were two (0.69 %) and four (1.38 %) of 290 samples containing a total amount of tetracyclines above and below the MRL, respectively.

The finding in the present study show that two compounds of the tetracycline group were found in the same sample and perhaps goes in line with other study's finding that the combination of antimicrobials in VMPs is very common and

imprudently used in the country, even two or more substances with the same pharmacologic characteristics are combined in a VMP. Once animals got sick, farmers buy VMPs and administer to the animals without any veterinary prescription and diagnosis (Boisseau, 2002). So, several VMPs may be used at the same time. This potentially results in a likelihood of applying compounds of the same drug family, for example tetracyclines, to an animal.

Ten screening-positive samples and 15 screening-inconclusive samples were not found to contain tetracycline compounds by HPLC analysis. The reason may be the well-known low specificity of the microbial growth inhibition test (Mitchell *et al.*, 1998; Gracey *et al.*, 1999; Nouws *et al.*, 1998; Chang *et al.*, 2000). This means that either other antibiotics than tetracyclines or/and that other non-specific antimicrobial factors may be present in the samples.

5.1.3. Experimental design and outcomes of the study

This study followed a regular procedure in analysis of antibiotic residue: a screening followed by a confirmation test. The microbiological inhibition screening test using *Bacillus cereus* as a microbial test strain on agar test pH 6 showed its effectiveness in separating out a majority of the tetracycline negative samples. As in other studies, in a number of samples generating inhibition zones in the microbiological growth inhibition test, no tetracyclines were demonstrated by the confirmatory method (De Wasch *et al.*, 1998).

However, it would be more cost effective if several kinds of antibiotic residues were screened at the same time by using several test organisms and agar media with different pH levels such as four-plate-test method (FPT) (Gracey *et al.*, 1999), modified four-plate-test method (MFPT) (Chang *et al.*, 2000), five-plate test or STAR protocol (Gaudin *et al.*, 2004), and six-plate method (Myllyniemi *et al.*, 2001). The confirmation test by HPLC is a sophisticated method with a very high sensitivity and specificity, and a low detection limit, but expensive in identifying and quantifying tetracycline residues in pork.

The results of the present study somewhat reflect the problem of antibiotic residue in Hanoi. These were limited to only residues of tetracycline compounds in marketed pork in Hanoi area. They do not represent residues of antibiotics in animal products of the whole country. Further studies would provide a more comprehensive view on this matter.

Despite limitations of methods and results in this study, it is recommended that the microbiological inhibition screening test using *Bacillus cereus* on agar test pH 6 coupled with HPLC should be used widely to test tetracycline residues in pork in the country. A similar protocol may be used for testing these substances in other kinds of meat such as beef and chicken. There should be further studies on residues of other commonly used antibiotics such as penicillin, streptomycin, gentamicin, tylosin and sulfonamides in animal products in the city and the whole country.

5.2. Conclusion

1. Out of 290 marketed pork samples, fifteen (5.17 %) were positive and 16 (5.52 %) were inconclusive with the microbial growth inhibition test (a screening test with *Bacillus cereus* as a test strain on test agar pH 6). Out of the 15 screening-positive samples, five were found to contain tetracycline compounds with HPLC method, as well as one of the 16 screening-inconclusive samples. In the suburban regions there is a higher risk to obtain meat with tetracycline residues than in shops in the urban areas.
2. Residues of three compounds of the tetracycline group are found with HPLC method in six (2.07 %) out of 290 marketed pork samples with individual concentrations ranging from 51.77 to 167.40 $\mu\text{g}/\text{kg}$, and sum concentration in each sample ranging from 51.77 to 192.26 $\mu\text{g}/\text{kg}$. Two (0.69 %) of 290 samples contain a sum concentration exceeding the MRL.