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Sensitivity of Repeat Tuberculin Test with Bovine PPD, Seibert's Fraction A (SFA) and Avian PPD Tuberculins in Visible and Non-visible Lesion Reactor Cattle to HCSM Tuberculin

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Tuberculin(HCSM)反應 乳牛에 대한 PPD, SFA 및 PPD-A Tuberculin을 이용한 再檢査法의 敏感性

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抄 錄 : Tuberculin(HCSM) 반응유우 60두에 대하여 가열살균처리한 우결핵균에서 얻은 PPD-BS tuberculin을 이용한 추벽피내재검사법과 석탄산살균처리한 우결핵균에서 얻은 SFA tuberculin을 이용한 경측피내재검사법간의 민감성과 특이성을 비교하였다. 또한 SFA tuberculin과 현행 조형 PPD (PPD-A) tuberculin을 이용한 비교검사법의 가치를 판독기준에 따라 분석하였다.

병소우군에서 PPD-BS와 SFA tuberculin간에 민감성은 차이가 없었으나 무병소우와 감염우동거군에서 SFA tuberculin은 PPD-BS tuberculin에 비하여 비특이반응이 현저히 낮았다($P<0.01$).

SFA와 PPD-A를 이용한 비교검사법은 판독기준에 따라 가양성반응과 가음성반응에 크게 영향을 주었으며, 피내반응차이 4mm를 판독기준으로 할 때 병소우와 무병소우를 감별할 수 없었다.

이 연구에서 SFA tuberculin은 PPD-BS에 비하여 병소우에서 민감성간에는 차이가 없었으나 무병소우에서 특이성이 현저히 높았다는 점으로 보아 앞으로 HCSM tuberculin 반응우에 대한 재검사는 현행 PPD에서 SFA tuberculin으로 대체함으로써 비특이반응우를 더욱 감소시킬 수 있다는 것을 의미한다.

Introduction

The control of bovine tuberculosis by tuberculin test and slaughter policy in Korea has a history of over half a century.[17,25] The incidence of bovine tuberculosis was markedly reduced from 18.4% in 1940 to about 0.99% in 1960 and 0.13% in 1980 as a result of systematic tuberculin testing and removal of reactors.[25]

However, one of the current problems of tuberculin test and slaughter policy is that the more incidence of bovine tuberculosis is reduced, the more non-visible lesion(NVL) cases or false positive reactors are increased. About 80% of the tuberculin reactors were found to be tuberculous at post-mortem examination at the inception of the scheme in 1940. On the contrary, about 69.6% of the tuberculin reactor cattle were false posi-

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tive or NVL cases in 1976.[9]

In many countries, to minimize the proportion of NVL cases, one of two ancillary tuberculin test, a repeat caudal fold tuberculin test or a single intradermal comparative tuberculin test is commonly adopted.[13,20,27] The repeat caudal fold test with mammalian tuberculin is used in the United States of America, Canada and Australia, while the single intradermal comparative test with bovine porified protein derivative (PPD) tuberculin and avian PPD(PPD-A) is widely used in Europe, some Middle East and South American countries.[13]

The repeat caudal fold test certainly reduced many NVL cases in the nontuberculous herd.[8,9,13] However, it may emerge new problems such that animals may be sensitized with tuberculin antigens[31,33] or desensitized by repeated testing.[21,30] To deal with this problem, a repeat caudal fold test should be done after an interval of 30 to 60 days[13] or a neck intradermal test should be substituted for the caudal fold test.[20]

In Korea, for the routine diagnostic procedure, 0.1ml of bovine HCSM tuberculin containing 10,000 tuberculin units(TU) is injected into the right caudal fold and for the repeat caudal fold test, 0.1ml of bovine PPD (PPD-BS) tuberculin containing 10,000 TU injected into the left caudal fold in suspicious and sometimes positive reactor cattle after an interval of 30 days.

The non-specific cross-reactions or NVL cases in dairy cattle usually occurred by sensitization with mycobacteria other than *Mycobacterium bovis* such as *M. avium* complex and *M. paratuberculosis*.[26,32,34] However, the important causes of the high false positive errors or NVL problem in this country have not been clarified.

In countries where the false positive errors are resulting from avian type infections and paratube rculosis a single intradermal comparative test would be of great value to differentiate them.[13] Nevertheless, the comparative test is hardly accepted as a routine diagnositic procedure possibly because it is not easy to administer, it is laborious and time consuming to perform and expensive.

In the past three decades, many attempts have been made to prepare new purified tuberculins to distinguish tuberculous and nontuberculous animals by single intradermal test.[9,11,19,22,29] However, no more highly specific antigen other than the PPD tuberculin is available at this time.[12]

In the previous study, authors[7] confirmed that the Seibert's fraction A(SFA tuberculin)[9,22,29] made from the culture filtrates of *M. bovis* killed at 60°C for one hour gave much smaller non-specific reactions than did conventional heated PPD tuberculin.[18] Similar results were observed with the PPD tuberculins prepared from culture filtrates of *M. bovis* killed by phenol.[4,5]

However, the senstivity and specificity of the unheated SFA tuberculin in tuberculous and non-tuberculous animals was not extensively studied. The main purpose of present study was to compare the sensitivity and specificity between the heated bovine PPD tuberculin(PPD-BS), unheated SFA tuberculin and PPD-A tuberculin in tuberculous animals, infected animal herd and non-tuberculous animals.

Materials and Methods

Tuberculin reactor cattle: Tuberculin reactor cattle to the initial HCSM tuberculin test and repeat caudal fold test with PPD-BS tuberculin were used. All animals available came from three areas; 41 cattle came from 23 farms in Kyunggi-do, 21 cattle came from 18 farms in Seoul city and three cattle came from 3 farms in Chungnam-do. Of all 65 reactor cattle 14 came from two infected farms and other 51 came from 42 tuberculosis-free farms. Animals were transported to the Institute of Veterinary Research and rested for 30 to 60 days before the comparative test.

After reading the tuberculin reactions, all animals except 5 cattle were slaughtered and post-mortem examination was carried out. The tuberculin reactors and slaughtered animals were devided into three groups on the basis of post-mortem examination; 17 visible lesion cases or tuberculous group, 34 NVL cases or non-tuberculous group and infected farm group.

Tuberculin: Conventional heated PPD-BS was prepared according to the method described by Green.[16,18] The HCSM tuberculin was a product of the Institute of Veterinary Research, Anyang. The SFA tuberculin was prepared according to the method of Seibert[29] and others[8,22] except that the mycobacterial cultures were killed by phenol as in the previous paper.[5] The PPD-BS, SFA and HCSM tuberculins were prepared with *M. bovis* strain AN_5 and the PPD-A tuberculin was made with *M. avium* strain D_4.

Tuberculin test: For the initial tuberculin test, 0.1ml of the HCSM tuberculin containing about 10,000 TU was injected into the right caudal fold. For the repeat test, 0.1ml of the PPD-BS tuberculin containing about 10,000 TU was injected into the left caudal fold after an interval of 30 days. For the comparative test, 0.1ml of the SFA tuberculin containing 10,000 TU were injected intradermally in front of the left shoulder, in the center of the base of the neck and the PPD-A tuberculin containing 2,500 TU was injected in the corresponding place of the right side. The increase of skin fold thickness was measured with callipers before injection and at the appropriate time afterwards.

Results

Number of visible lesion and non-visible lesion cases: On the basis of the post-mortem examination, of 60 tuberculin reactor cattle, 17 (28.3%) animals had tuberclelike lesions and tentatively diagnosed as tuberculous animals and 43(71.6%) were NVL cases and tentatively diagnosed as nontuberculous animals.

Sensivity of PPD-BS, SFA and PPD-A tuberculin: The sensitivity of PPD-BS, SFA and PPD-A tuberclins were compared in visible lesion cases, infected animal herd and non-visible lesion cases.

As shown in Table 1, the mean increases of skin fold thickness to PPD-BS in the caudal fold test and SFA tuberculin in the neck test were 10.4 ±5.5mm and 10.0±5.9mm in visible lesion cases and 10.1±3.3mm and 7.8±4.0mm in infected animal herd, respectively. The mean skin reactions to PPD-BS and SFA tuberculins were respectively 7.0±2.0mm and 3.2±2.3mm in NVL cases. Statistically the difference of mean skin reactions between PPD-BS and SFA tuberculins were not significant, but the SFA tuberculin gave much smaller reactions than the PPD-BS tuberculin did in infected animal herd($P<0.05$) and NVL cases($P<0.01$).

In visible lesion cases and infected animl herd, the skin reactions to SFA tuberculin were 3.9 to 4.7mm greater than to the PPD-A tuberculin, whereas in NVL cases the skin reaction to the PPD-A tuberculin was 0.9mm greater than to the SFA tuberculin.

The mean skin reactions to PPD-A tuberculin in visible lesion cases, infected animal herd and

Table 1. Sensitivity of bovine PPD, SFA and PPD-A tuberculin in visible lesion case, infected animal herd and non-visible lesion case groups

Animal group	No. of animals tested	Repeated caudal fold test PPD-BS	Comparative test in the neck					
			p	SFA	p	PPD-A	p	Difference(mm)
Visible lesion	17	10.4±5.5#	—	10.0±5.9	—	5.3±2.8	—	>4.7
	p	—		NS		**		
Infected herd	14	10.1±3.3	NS	7.8±4.0	NS	3.9±1.6	NS	>3.9
	p	—		*		**		
Non-visible lesion	34	7.0±2.0	**	3.2±2.3	**	4.1±2.0	NS	<0.9
	p	—		**		**		

Expressed as mean increase in the skin fold thickness in mm.
* $p<0.05$, **$p<0.01$, NS, not significant by student t test.

Tbale 2. Errors at different levels of interpretation of SFA tuberculin test in visible and non-visible lesion groups

Positive criteria*	False positive in 31 NVL cases(%)		False negative in 17 visible lesion cases(%)	
	48	72hr**	48	72%
>1 mm	22.5	29.0	29.5	35.3
>2 mm	29.0	29.0	29.5	35.3
>3 mm	25.8	19.3	29.5	41.2
>4 mm	12.9	19.3	47.1	41.2
>5 mm	9.6	0.0	53.0	58.9

* Difference in increase of the skin fold thickness between sensitivity to SFA tuberculin and PPD-A tuberculin.

** Reading time.

NVL cases were 5.3±2.8mm, 3.9±1.6mm and 4.1 ±2.0mm, respectively and no significant difference between experimental groups was observed.

Errors at different levels of interpretation of the comparative test: A positive criterion of increase of the skin fold thickness was compared in respect of sensitivity and specificity to differentiate visible lesion cases and NVL cases.

The differences between the result at the 48 hour and 72 hour reading were not significant and the 48 hour readings were adopted here. As shown in Table 2, if a positive criterion of 4mm were adopted, the false positive rate was 12.9%, whereas the false negative would be 47.1%. If the positive criterion of 5mm were adopted, the false positive reduced to 9.6% but the false negative markdely increased to 53.0%.

As shown in Table 3, if a positive criterion of 4mm is adoped, the sensitivities of the SFA tuberculin and PPD-A tuberculin to differentiate between visible lesion cases and NVL cases were 52.9

Table 3. Sensitivity at different levels of interpretation in the comparative test with SFA tuberculin (10,000 TU) and PPD-A tuberculin (2,500 TU) in visible and non-visible lesion groups

Difference criteria* (mm)	17 Visible lesion cases, dominant to SFA(%)	31 Non-visible lesion cases, dominant to PPD-A(%)
> 1	12(70.5)	16(51.6)
> 2	12(70.5)	11(35.4)
> 3	12(70.5)	4(12.9)
> 4	9(52.9)	3(9.6)
< 5	8(47.0)	1(3.2)

* Difference in increase of the skin fold thickness between the sensitivity to SFA tuberculin(10,000 TU) and avian tuberculin(2,500 TU).

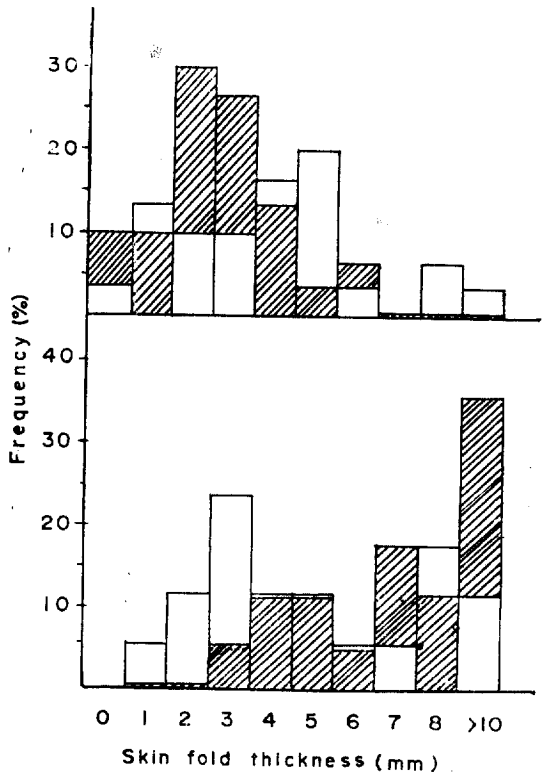

Fig. Frequency distribution of skin fold thickness to SFA tuberculin(■) and PPD-A tuberculin(□) in non-visible lesion cases(above) and visible lesion cases(bellow).

% and 9.6%, respectively. A lowering of the difference criterion from 4mm to 2mm increases the sensitivity of SFA stuberculin in visible lesion cases to 70.5% and of PPD-A tuberculin in NVL cases to 35.4%, repectively.

Frequency distribution of the skin reactions to SFA tuberculin and PPD-A tuberculin in visible and NVL cases: Figure 1 shows the frequency distribution of the increase of skin fold thickness of the comparative test in visible lesion cases.

Although the results of comparative test, under the condition of this experiment, was entirely unsatisfactory to differentiate between visible and non-visible lesion cases, the high frequency distribution of the skin fold thickness greater than 10 mm to SFA tuberculin appeared in visible lesion cases, where as the skin reaction less than 4mm to the SFA tuberculin appeared in NVL cases.

Discussion

The comparative test using mammalian and avian tuberculins would be a promising tool for an animal population with a non-specific problem resulting from avian mycobacterial infection and paratuberculosis[13,20,27]. The repeat caudal fold test, however, would be more widely used in countries where the incidence of avian infections or paratuberculosis is low[14,15]. In the comparative test, if the false positive reaction is absent and reactions to mammalian tuberculin are not more than 4mm greater than to avian tuberculin, initial tuberculin reactor animals are usually retained and retested[27].

In this study, if a positive criterion of 4mm were adoped for single intradermal repeat test with the SFA tuberculin, the false positive and false negative errors were 12.9% and 47.1%, respectively. A lowering of the positive criterion from 4mm to 2mm reduced the false negative error from 47.1% to 29.5%, but increased the false positive error from 12.9% to 29.0%. If a positive criterion of 5mm at 72 hour reading were adoped, the false positive error reduced to zero but the false negative error increased to 58.9%. This result indicates that the repeat test is also unsatisfactory to differentiate between visible and non-visible lesion cases. This is coincident with another worker[14].

In the comparative test with the SFA tuberculin (10,000 TU) and the PPD-A tuberculin (2,500 TU), of 31 NVL cases 16(51.6%) were 1mm greater to avian tuberculin than to bovine tuberculin and only 3(9.6%) were 4mm greater to avin tuberculin, respectivley. In visible lesion cases, of 17 animals, 12(70.5%) were 3mm greater to SFA tuberculin than to avian tuberculin and 9(52.9%) were 4mm greater. This result stggests that the comparative test is entirely unsatisfactory in this country, and it may indicate that the avian mycobacterial infections would not be an important cause of the false positive errors in this country.

Lee *et al*[24] isolated 25 strains of mycobacteria from 150 lymph nodes of thoracic cacity randomley collected from the native Korean cattle and 16 (64.0%) were *M. avium* complex. Separatly, authors[6] isolated 70 strains of mycobacteria from tubercle-like lesions and lymph nodes in the thoracic cavity, abdominal cavity, head and udder of 76 tuberculin reactor dairy cattle and 33(47.1%) were *M, bovis*, 16(22.8%) were *M. gordonae*, 15 (21.4%) were *M. terrae* complex, 5(7.1%) were *M. avium* complex and 2(2.8%) were *M. fortuitum* complex. These results indicate that *M. avium* infections would be included in the false positive reactors in this country. However, without further study on the isolation of *M. avium* complex from NVL cases, it is imposible to say at this stage whether avian tuberculosis in dairy cattle is an important cause of the false positive problems in this country.

Authors[3] also conducted intradermal johnin test in 833 dairy cattle and detected 8(0.9%) positive reacrors and 5(62.5%) were cenfirmed by complement fixation test. This result suggests that *M. paratuberculosis* would also be distributed in dairy cattle. Recently Jeon (personal communication) isolated *M. paratuberculosis* from dairy cattle in this country. These results suggests that *M. par-*

atuberculosis would be a casue of false positive errors in this country.

In the previous studies on the isolation of avian mycobacteria in cattle in this country, it presumed that *M. avium* would be more widely distributed in the Korean native cattle than in dairy cattle. A low incidence of avian mycobacterial infection in pigs was also revealed in this country[11].

As shown in Figure 1, in the frequency distribution of skin reactions to the SFA tuberculin, the fractions greater than 10mm appeared with much higher frequency in visible lesion cases, while the reactions less than 4mm appeared with migher frequency in NVL cases. This result may suggest that a positive criterion of 5mm would be adopted, despite the increase of the false negative, to differntiate the NVL cases or non-tuberculous animals from tuverculous animals.

In this study, no significant difference between mean increase of skin reactions to PPD-BS and SFA tuberculin was observed in visible lesion group, but the SFA tuberculin gave much smaller reactions than PPD-BS tuberculin did either in infected animal herd ($P<0.05$) or nonvisible lesion group ($P<0.01$).

This result indicates that the SFA tuberculin would be a little more specific than conventional PPD-BS tuberculin and would be of great value for repeat test. This result is coincident with the result of the previous works[9,22].

Conclusion

The sensitivity and specificity of heated bovine purified protein derivative (PPD-BS) tuberculin in the caudal fold test and unheated bovine Seibert's fraction A(SFA) tuberculin in the neck test was compared, and a comparative tuberculin test using the SFA tuberculin and avian tuberculin (PPD-A) was carried out in 60 tuberculin reactor cattle in respect of its efficiency in differentiating between visible lesion cases and non-visible lesion(NVL) cases in this country.

There was not significant difference between the heated PPD-BS tuberculin test and the unheated SFA tuberculin test in visible lesion cases, but the SFA tuberculin gave much smaller non-specific reactions than the PPD-BS tuberculin did in infected animal herd ($P<0.05$) and in NVL cases ($P<0.01$). However the skin reactions to PPD-A tuberculin between experimental groups were not significant.

For the comparative test, if a positive criterion of 4mm were adopted, the false positive and fase negative errors were 12.9% and 47.1%, respectively. A lowering of the positive criteria reduced the false negative errors, but increased the false positive errors.

This result indicated that the comparative test, in the neck as ancillary test, with the SFA tuberculin and the PPD-A tuberculin was unsatisfactory to distinguish between vissible and NVL cases, but for the repeat test the unheated SFA tuberculin would be substituted for the heated PPD-BS tuberculin.

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