动物伦理审查同意书

Affidavit of Approval of Animal Ethical and Welfare

编号 Approval No.	GSAU-AEW-2014-0003

本《动物实验方案》经过实验动物伦理委员会审核，符合动物保护、动物福利和伦理原则，符合国家实验动物福利伦理的相关规定。方案的相关信息如下：

The animal use protocol listed below has been reviewed and approved by the Animal Ethical and Welfare Committee (AEWC).

<table>
<tr><td>研究名称
Protocol Title</td><td colspan="6">奶牛乳腺炎来源肠球菌的毒力、抗药性及其致病性的研究
Prevalence of virulence profile and antibiotic susceptibility of pathogenic enterococci isolated from mastitic cows</td></tr>
<tr><td>申请人
Applicant</td><td>武小虎
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Title/Degree</td><td>博士
PhD.</td><td>邮箱
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<tr><td>课题负责人
Principle Investigator(PI)</td><td>赵兴绪
Xingxu Zhao</td><td>职称/学位
Title/Degree</td><td>教授
Prof.</td><td>邮箱
Email</td><td colspan="2">zhaoxx@gsau.edu.cn</td></tr>
<tr><td>院系（部门）
Department</td><td colspan="3">动物医学院
College of Veterinary Medicine</td><td>申请日期
Application date</td><td>2014.03.10</td></tr>
<tr><td>动物种系
Species or Strains</td><td colspan="3">小鼠
Kunming mice</td><td>动物数量
Quantity</td><td>48</td></tr>
<tr><td>计划执行时间
Period of Protocol</td><td colspan="2">2014.05.01-2015.04.30</td><td>实验动物编号
Number of Animal permit</td><td colspan="3">No. SCXK (Gan)：No</td></tr>
<tr><td>审查意见
Results of inspection</td><td colspan="6">☑符合动物福利伦理要求，可以进行实验 Agree
☐调整方案后，可以进行实验 Agree after modify</td></tr>
</table>

甘肃农业大学动物医学院实验动物伦理委员会

Animal Ethical and Welfare Committee of College of Veterinary Medicine of Gansu Agricultural University

主席 (Chairman): Du Xiaohua

日期 (Date): 2014.3.12

Antimicrobial agents gradients and the standard ranges of each drug*

Antimicrobial agents	Antimicrobial resistance assessment standards			Antimicrobial gradients prepared in the 96 well plates (mg/μL)										positive control	quality control
	sensitive	intermediate	resistant		1	2	3	4	5	6	7	8	9	10	11	12
Pen	≤8	-	≥16	A	128	64	32	16	8	4	2	1	0.5	0.25	0	0
Amp	≤8	-	≥16	B	128	64	32	16	8	4	2	1	0.5	0.25	0	0
Van	≤4	8-16	≥32	C	128	64	32	16	8	4	2	1	0.5	0.25	0	0
Tet	≤4	8	≥32	D	128	64	32	16	8	4	2	1	0.5	0.25	0	0
Cip	≤1	2	≥4	E	32	16	8	4	2	1	0.5	0.25	0.13	0.06	0	0
Gen	500 mg/μL			F	500	500	5000									0
Str	1000 mg/μL			G	1000	1000	1000									0

1. For wells of 1 to 12 in column A to column E, antimicrobials were added by doubling dilution method as shown in the corresponding wells. Briefly, firstly added 100 μL of cultural medium were added to well 1 to well 12. Then, 2× antimicrobial agents(2 times concentration for well 1) sulution were added to well 1 and mixed well. Pepitting half of the solution to the next well untill well 10. Well 11 and well 12 were served as positive and quality control, respectively.

2. 100 μL of 0.5 McFarland turbidity overnight bacteria medium were added to well 1 to well 11, and well 12 was only filled with 100 μL cultural medium. The plates were then mixed well and incubated at 37 °C enviroment for 18 to 24 hours according to the NCCLS standards*.

* Cockerill, F.R., Clinical, Institute, L.S., 2012b. Performance standards for antimicrobial susceptibility testing: twenty-second informational supplement. National Committee for Clinical Laboratory Standards.

Virulence gene detection and 16S rDNA identification of the 60 enterococcal isolates

	Esp	*GelE*	*Ccf*	*CylA*	*Asa1*	*Ace*	*Agg*	Virulence count	asa1^{+} ccf^{+} gelE^{+}	Identification	
ATCC51299	+	+	+	+	+	+	+	7	+++	*E. faecalis*	GVI
ATCC29212	-	+	+	+	+	+	-	5	+++	*E. faecalis*	GVI
MS4	-	+	-	-	+	+	-	3		*E. hirae*	GVI
MS5	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS6	-	-	-	-	+	-	-	1		*E. hirae*	NGVI
MS7	-	+	-	-	+	-	-	2		*E. hirae*	GVI
MS8	-	-	-	-	+	-	-	1		*E. hirae*	NGVI
MS9	+	-	-	-	+	-	-	2		*E. hirae*	NGVI
MS10	-	-	-	-	+	-	-	1		*E. hirae*	NGVI
MS11	-	+	+	+	+	-	-	4	+++	*E. hirae*	GVI
MS12	-	+	+	-	+	+	-	4	+++	*E. hirae*	GVI
MS13	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS14	-	+	-	-	+	-	-	2		*E. hirae*	NGVI
MS15	-	+	-	-	+	-	-	2		*E. hirae*	NGVI
MS16	-	+	+	+	+	-	-	4	+++	*E. hirae*	GVI
MS17	-	-	-	-	+	-	-	1		*E. hirae*	NGVI
MS18	-	-	+	-	+	-	-	2		*E. hirae*	NGVI
MS19	-	-	-	-	+	-	-	1		*E. hirae*	NGVI
MS20	-	-	-	-	+	-	-	1		*E. hirae*	NGVI
MS21	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS22	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS23	-	+	+	+	+	-	-	4	+++	*E. hirae*	GVI
MS24	-	+	-	-	+	-	-	2		*E. hirae*	NGVI
MS25	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS26	+	+	+	-	+	+	-	5	+++	*E. hirae*	GVI
MS27	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI

MS28	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS29	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS30	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS31	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS32	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS33	-	+	+	-	+	+	-	4	+++	*E. hirae*	GVI
MS34	-	+	-	+	+	-	-	3		*E. hirae*	GVI
MS35	+	+	+	-	+	+	-	5	+++	*E. hirae*	GVI
MS36	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS37	-	+	+	-	+	-	-	3	+++	*E. hirae*	GVI
MS38	-	+	+	-	+	+	-	4	+++	*E. hirae*	GVI
MS39	-	+	+	-	+	+	-	4	+++	*E. hirae*	GVI
MS40	+	+	+	-	+	-	-	4	+++	*E. hirae*	GVI
MS41	-	+	+	-	+	+	-	4	+++	*E. hirae*	GVI
MS42	-	+	+	+	+	-	-	4	+++	*E. hirae*	GVI
MS43	-	-	+	-	+	-	-	2		*E. hirae*	NGVI
MS44	-	-	+	-	+	+	-	3		*E. hirae*	GVI
MS45	-	-	-	-	+	-	-	1		*E. durans*	NGVI
MS46	+	+	-	-	+	+	-	4		*E. durans*	GVI
MS47	-	+	+	-	+	-	-	3	+++	*E. mundtii*	GVI
MS48	-	+	+	-	+	-	-	3	+++	*E. mundtii*	GVI
MS49	-	+	-	-	+	-	-	2		*E. faecium*	NGVI
MS50	-	-	+	-	+	-	-	2		*E. faecium*	NGVI
MS51	-	+	+	-	+	-	-	3	+++	*E. faecium*	GVI
MS52	-	+	-	-	+	+	-	3		*E. faecium*	GVI
MS53	-	-	-	-	+	-	-	1		*E. faecium*	NGVI
MS54	-	+	+	-	+	-	-	3	+++	*E. faecium*	GVI
MS55	-	+	-	-	+	-	-	2		*E. faecium*	NGVI
MS56	-	-	-	-	+	-	-	1		*E. faecium*	NGVI

MS57	+	+	+	-	+	-	-	4	+++	*E. faecium*	GVI
MS58	-	+	+	-	+	-	-	3	+++	*E. faecium*	GVI
MS59	-	+	+	-	+	-	-	3	+++	*E. faecium*	GVI
MS60	-	+	+	-	+	-	-	3	+++	*E. faecium*	GVI
MS61	-	+	-	-	+	-	-	2		*E. faecium*	NGVI
MS62	+	+	+	-	+	-	-	4	+++	*E. faecium*	GVI
MS63	-	-	+	-	+	-	-	*2*		*E. faecium*	NGVI

"+++", stands for the isolates was poritive to asa1, ccf and gelE positve.

GVI, genetically virolent isolates. NGVI, non-genetically virulent isolates.