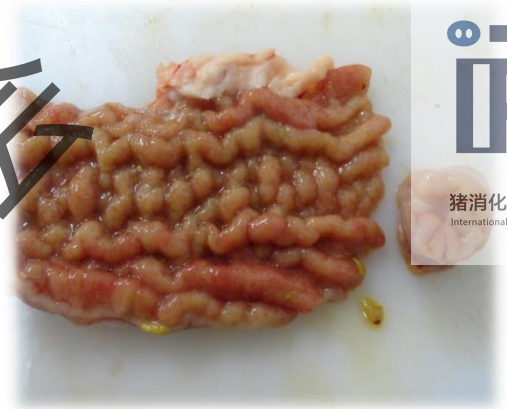
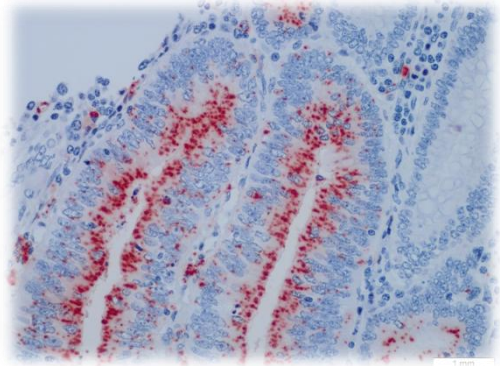
Proliferative enteropathy: vaccine control and management strategies
增生性肠炎（回肠炎）：疫苗控制和管理策略

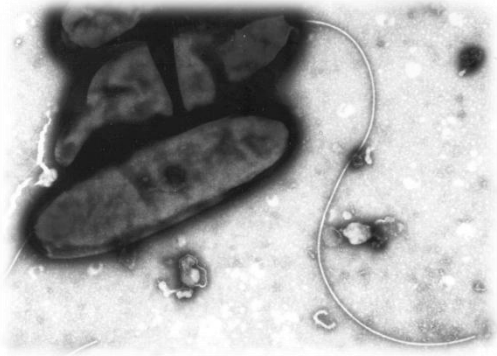
Roberto Maurício Carvalho Guedes 罗伯托-毛里西奥-卡瓦略-格德斯

Universidade federal de Minas Gerais

巴西 米纳斯吉拉斯联邦大学







UFMG

猪消化道疾病国际研讨会
International Pig Digestive Diseases Symposium

Roberto Guedes – Professor (1995) 罗伯托-格德斯教授（1995）

Universidade Federal de Minas Gerais - Veterinary SchoolDepartment Clinic and Surgery

Animal Pathology Research Laboratory

米纳斯吉拉斯联邦大学兽医学院动物病理学研究实验室

5.375.579 m²

Management strategies管理策略

- Risk Factors 风险因素

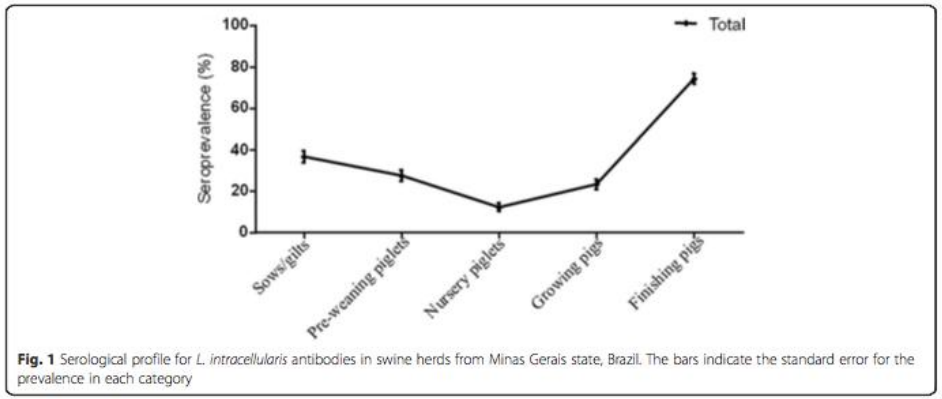


Fig. 1 Serological profile for *L. intracellularis* antibodies in swine herds from Minas Gerais state, Brazil. The bars indicate the standard error for the prevalence in each category

Fig. 1 Serological profile for L. intracellularis antibodies in swine herds from Minas Gerais state, Brazil. The bars indicate the standard error for the prevalence in each category 米纳斯吉拉斯州猪群细胞内抗体的血清学特征。条形图表示每个类别中流行率的标准误差。

BMC Veterinary Research
RESEARCH ARTICLE Open Access

Serological profile, seroprevalence and risk factors related to Lawsonia intracellularis infection in swine herds from Minas Gerais State, Brazil 巴西米纳斯吉拉斯州猪群的血清学概况、血清流行率以及与胞内劳森菌感染有关的风险因素

Talita Pilar Resende[1], Carlos Eduardo Real Pereira[1], Michelle de Paula Gabardo[1], João Paulo Amaral Haddad[2], Zélia Inês Portela Lobato[1] and Roberto Maurício Carvalho Guedes[1*]

Resende et al. BMC Veterinary Research (2015) 11:306
DOI 10.1186/s12917-015-0618-z

Table 3 Linear regression results for risk factors. Only the "cleaning before disinfecting" showed a significant association to the L. intracelularis herd level seropositivity
表 3 风险因素的线性回归结果。只有 "消毒前清洁 "与 牛群胞内劳森菌血清阳性率有显著关联

Variable 变量	*P* value	Regression 地区 coefficient	Standard error 标准差	CI min	CI max
New born assistance at farrow and piglets' first colostrum intake 人工助产和仔猪第一次吃初乳	0.22	0.17	0.14	−0.11	0.45
Crossfostering management 交叉寄养管理	0.12	-.0.70	0.05	−0.17	0.03
Cleaning before disinfection 消毒前清洁	0.01	−0.04	0.02	−0.07	−0.01
Number of disinfectants used 消毒剂使用数量	0.11	0.01	0.04	−0.07	0.09

CI–Confidence interval

Quaternary ammonium compounds (Roccal-D PlusR, DC&RR, SynergizeTM), aldehydes (DC&RR, SynergizeTM), oxidizing agents (VirkonR S), biguanindes (NolvasanR Solution), phenol (Tek-Trol R), iodine (Cetridine), chlorine, potassium peroxymonosulfate, phosphate compounds (StalosanR F), and sulfate compounds inactivate *L. intracellularis*季铵盐化合物、醛类、氧化剂、双胍类、苯酚、碘、氯、过硫酸锰钾、磷酸盐化合物和硫酸盐化合物可灭活细胞内嗜酸乳杆菌。

Management strategies

- Risk Factors

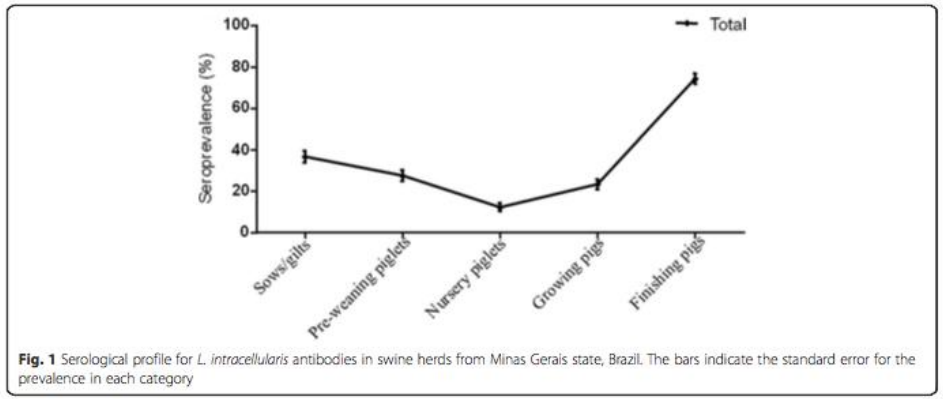


Fig. 1 Serological profile for *L. intracellularis* antibodies in swine herds from Minas Gerais state, Brazil. The bars indicate the standard error for the prevalence in each category

BMC Veterinary Research

RESEARCH ARTICLE Open Access

Serological profile, seroprevalence and risk factors related to *Lawsonia intracellularis* infection in swine herds from Minas Gerais State, Brazil

Talita Pilar Resende[1], Carlos Eduardo Real Pereira[1], Michelle de Paula Gabardo[1], João Paulo Amaral Haddad[2], Zélia Inês Portela Lobato[1] and Roberto Maurício Carvalho Guedes[1*]

Resende *et al. BMC Veterinary Research* (2015) 11:306
DOI 10.1186/s12917-015-0618-z

Table 3 Linear regression results for risk factors. Only the "cleaning before disinfecting" showed a significant association to the L. intracelularis herd level seropositivity

Variable	P value	Regression coefficient	Standard error	CI min	CI max
Newborn assistance at farrow and piglets' first colostrum intake	0.22	0.17	0.14	−0.11	0.45
Cross fostering management	0.12	-0.70	0.05	−0.17	0.03
Cleaning before disinfection	0.01	−0.04	0.02	−0.07	−0.01
Number of disinfectants used	0.11	0.01	0.04	−0.07	0.09

CI – Confidence interval

Quaternary ammonium compounds (Roccal-D PlusR, DC&RR, SynergizeTM), aldehydes (DC&RR, SynergizeTM), oxidizing agents (VirkonR S), biguanindes (NolvasanR Solution), phenol (Tek-Trol R), iodine (Cetridine), chlorine, potassium peroxymonosulfate, phosphate compounds (StalosanR F), and sulfate compounds inactivate *L. intracellularis*

Prevention and control 预防和控制

- **Treatment:**治疗
- **Injectable (clinically sick animals), water soluble and in-feed medication**
  - 注射剂（用于临床病猪）、水溶性和饲料中的药物。
- **Prevention:**预防
  - **Continuous use of in-feed medication (Restrictions and cost)** 持续使用饲料中的药物（限制和成本）。
  - **“Immunological windows”** “免疫学窗口”
    - **Pulse medication (7 to 14 days), interval (3 to 4 weeks) (beginning of grow-finishing), new pulse medication**
      脉冲式给药（7至14天），间隔（3至4周）（生长-育肥初期），新的脉冲式给药。
    - **Single pulse medication, based on herd seroprofile (or oral fluid)**
      基于群体血清学特征（或口腔液体）的单次脉冲给药。
    - **“Window” at the nursery** 保育舍的“窗口”。
- **Alternatives – Nutraceuticals** 替代品 - 营养保健品。
  - Vaccine – Modified-live (Enterisol® Ileitis, Boehringer Ingelheim – Oral/Nasal) Nutraceuticals 疫苗 - 改良活疫苗（如口服/鼻喷）。
  - Vaccine - Killed (Porcilis Ileitis, Merck Animal Health– IM) 疫苗 - 灭活疫苗（如肌肉注射）。
  - Vaccine new technologies ... 疫苗新技术……

Isolation and in vitro antimicrobialsusceptibility of porcine Lawsoniaintracellularis from Brazil and Thailand巴西和泰国的猪腹腔劳森氏菌的分离和体外抗菌药敏感性

BMC Microbiology

Suphot Wattanaphansak[3†], Carlos Eduardo Real Pereira[1†], Wenika Kaenson[3], Pornchalit Assavacheep[3], Rachod Tantilertcharoen[3], Talita Pilar Resende[2], Javier Alberto Barrera-Zarate[1], Juliana Saes Viaça de Oliveira-Lee[1], Ulrich Klein[4], Connie Jane Gebhart[2] and Roberto Maurício Carvalho Guedes[1*]

Table 1 Extracellular and intracellular MIC endpoints for 5 antimicrobials against two Brazilian L. intracellularis isolates. The bacteria were prepared independently and tested twice. The endpoint was obtained from 3 replicates of each passage. Minimum Inhibitory Concentration (MIC) μg/ml 5 种抗菌药物对两种巴西胞内劳森氏菌的细胞外和细胞内 MIC 终点。细菌独立制备并测试两次。每个菌株的终点由 3 个重复菌株得出。最低抑菌浓度 (MIC) μg/ml

Antimicrobials 抗菌药	**Minimum Inhibitory Concentration (MIC) μg/ml 最低抑菌浓度**			
	BRPHE01_E5Passages 13–14		**BRPHE02_E8Passages13–14**	
	Intracellular activity 胞内活性	**Extracellular activity 胞外活性**	**Intracellular activity 胞内活性**	**Extracellular activity 胞外活性**
1. Chlortetracycline 金霉素	32–64	32–64	8–16	64
2. Lincomycin 林可霉素	> 128	> 128	> 128	> 128
3. Tiamulin 泰妙菌素	≤0.125	1–2	1–2	0.5
4. Tylosin 泰乐菌素	2–8	> 128	2	16–32
5. Valnemulin 沃尼妙林	≤0.125	≤0.125	≤0.125	≤0.125

Wattanaphansak et al. BMC Microbiology (2019) 19:27
https://doi.org/10.1186/s12866-019-1397-7

Prevention and control 预防和控制

- **Treatment:**治疗
- **Injectable (clinically sick animals), water soluble and in-feed medication**
  - 注射剂（用于临床病猪）、水溶性和饲料中的药物。
- **Prevention**:预防
  - **Continuous use of in-feed medication (Restrictions and cost)** 持续使用饲料中的药物（限制和成本）。
  - **“Immunological windows”** “免疫学窗口”
    - **Pulse medication (7 to 14 days), interval (3 to 4 weeks) (beginning of grow-finishing), new pulse medication**
      脉冲式给药（7至14天），间隔（3至4周）（生长-育肥初期），新的脉冲式给药。
    - **Single pulse medication, based on herd seroprofile (or oral fluid)**
      基于群体血清学特征（或口腔液体）的单次脉冲给药。
    - **“Window” at the nursery** 保育舍的“窗口”。
- **Alternatives – Nutraceuticals** 替代品 - 营养保健品。
  - Vaccine – Modified-live (Enterisol® Ileitis, Boehringer Ingelheim – Oral/Nasal) Nutraceuticals 疫苗 - 改良活疫苗（如口服/鼻喷）。
  - Vaccine - Killed (Porcilis Ileitis, Merck Animal Health– IM) 疫苗 - 灭活疫苗（如肌肉注射）。
  - Vaccine new technologies …. 疫苗新技术……

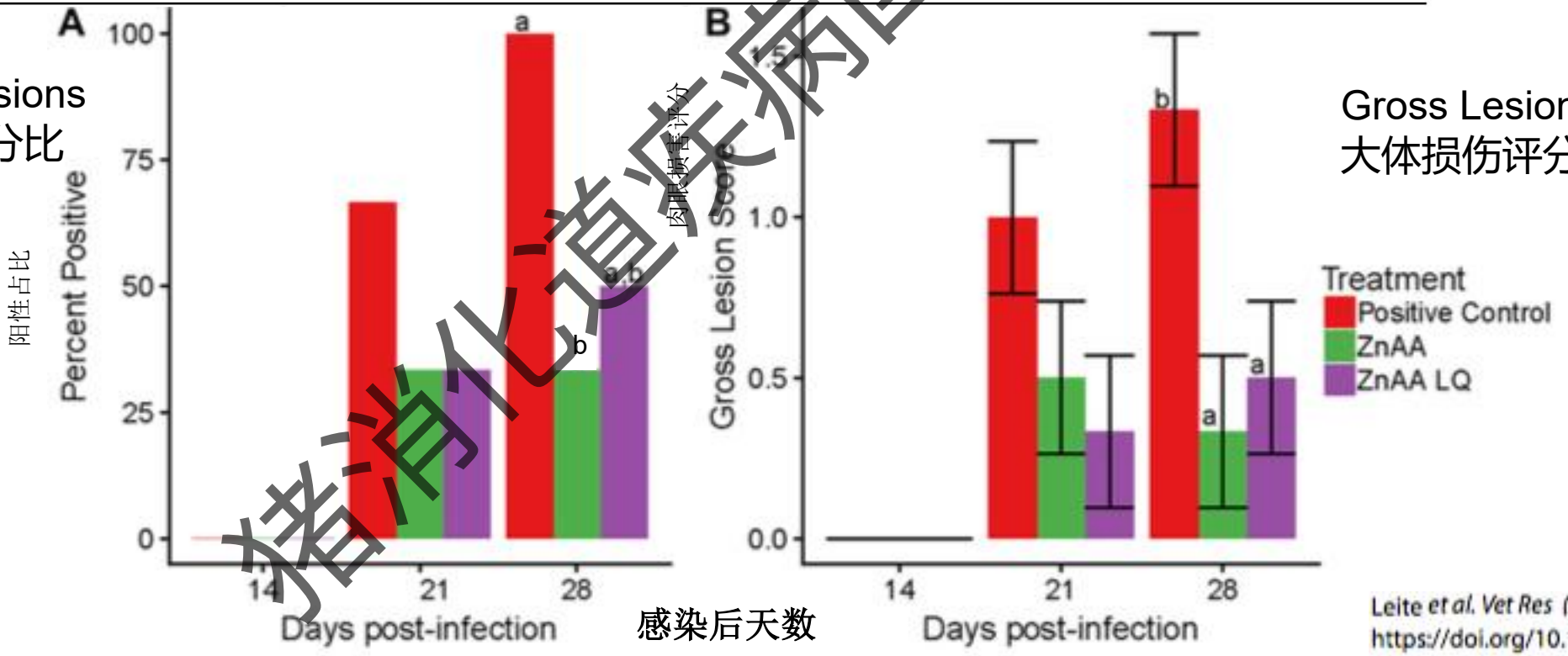
The effects of zinc amino acid complex supplementation on the porcine host responseto Lawsonia intracellularis infection

补充氨基酸锌复合物对猪宿主的细胞内劳森氏杆菌感染反应的影响

Table 1 Animal treatments 动物处理

Treatment 治疗	Pens/treatmen 每组6个笼子	Pigs/pen	Pigs/treatment 每笼三头	Zn level (ppm) and source 每组共18头	Challenge 挑战
Positive control 阳性对照	6	3	18	125, SO_4	Yes 是
ZnAA in feed 饲料中添加锌氨基酸复合物	6	3	18	75 SO4 + 50 Availa ZN	Yes 是
ZnAALQ in water 水中添加锌氨基酸液体	6	3	18	125 SO_4 + Availa ZN LQ	Yes 是
Negative control 阴性对照	6	3	18	125, SO_4	No 否

% Gross Lesions
大体损伤百分比



Gross Lesions score
大体损伤评分

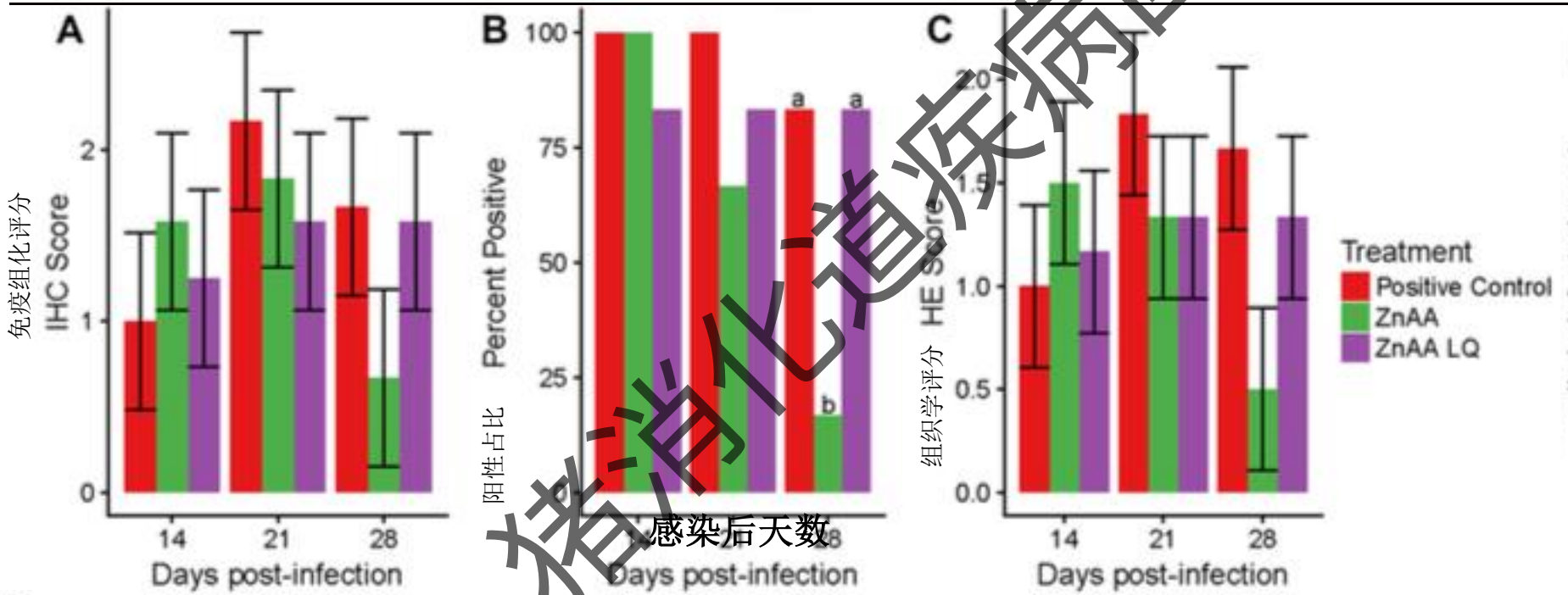
Leite et al. Vet Res (2018) 49:88
https://doi.org/10.1186/s13567-018-0581-3

The effects of zinc amino acid complex supplementation on the porcine host responseto Lawsonia intracellularis infection

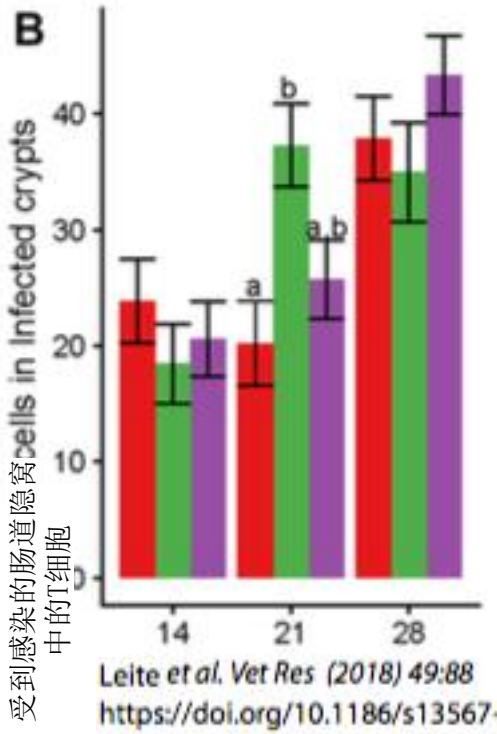
补充氨基酸锌复合物对猪宿主的细胞内劳森氏杆菌感染反应的影响

Table 1 Animal treatments 动物处理

Treatment 治疗	Pens/treatmen每组6个笼子	Pigs/pen	Pigs/treatment 每笼三头	Zn level (ppm) and source 每组共18头	Challenge 挑战
Positive control 阳性对照	6	3	18	125, SO_4	Yes 是
ZnAA in feed 饲料中添加锌氨基酸复合物	6	3	18	75 SO4 + 50 Availa ZN	Yes 是
ZnAALQ in water 水中添加锌氨基酸液体	6	3	18	125 SO_4 + Availa ZN LQ	Yes 是
Negative control 阴性对照	6	3	18	125, SO_4	No 否



% histologic lesions 组织学病变百分比



饲料中添加枯草芽孢杆菌益生菌可减轻胞内劳森菌的脱落和病变

Bacillus pumilus probiotic feed supplementation mitigates *Lawsonia intracellularis* shedding and lesions

Tanja Opriessnig[1,2*], Anbu K. Karuppannan[2], Dana Beckler[3], Tahar Ait-Ali[1], Ana Cubas-Atienzar[1] and Patrick G. Halbur[2]

VETERINARY RESEARCH
Opriessnig et al. Vet Res (2019) 50:85
https://doi.org/10.1186/s13567-019-0696-1

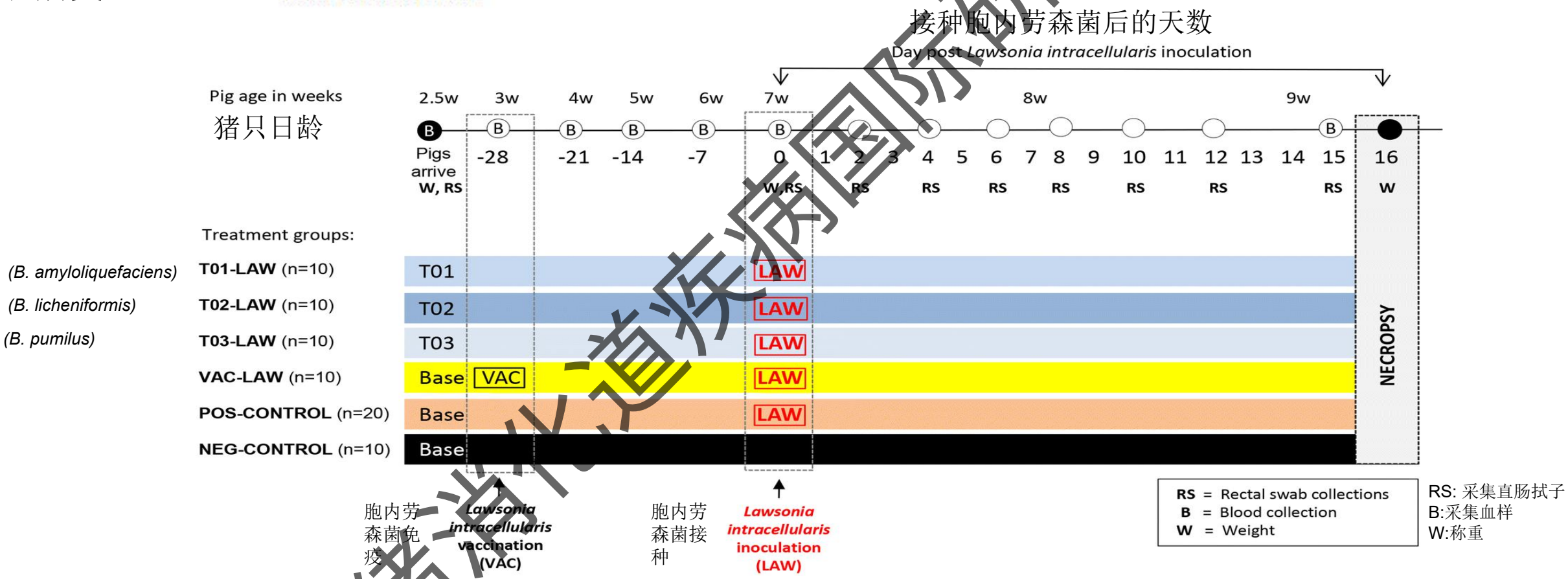


Figure 2 Experimental design including time lines and major sampling events. Base, base diet; T01, base diet with Bacillus amyloliquefaciens, TO2, base diet with Bacillus licheniformis, and T03, base diet with Bacillus pumilus. 实验设计包括时间线和主要取样事件。Base：基础日粮；T01：添加淀粉芽孢杆菌的基础日粮；TO2：添加地衣芽孢杆菌的基础日粮；T03：添加枯草芽孢杆菌的基础日粮。

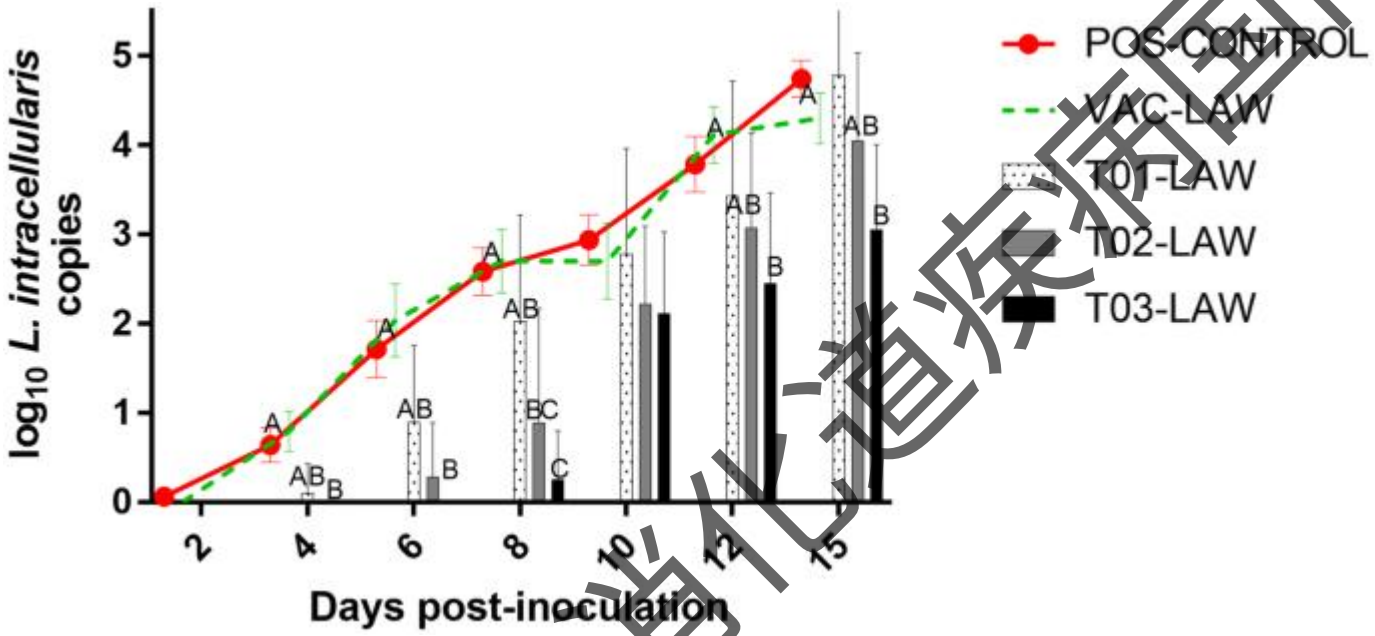
UFMG

Bacillus pumilus probiotic feed supplementation mitigates *Lawsonia intracellularis* shedding and lesions

Tanja Opriessnig[1,2*], Anbu K. Karuppannan[2], Dana Beckler[3], Tahar Ait-Ali[1], Ana Cubas-Atienzar[1] and Patrick G. Halbur[2]

饲料中添加枯草芽孢杆菌益生菌可减轻胞内劳森菌的脱落和病变

VETERINARY RESEARCH
Opriessnig et al. Vet Res (2019) 50:85
https://doi.org/10.1186/s13567-019-0696-1



(*B. amyloliquefaciens*)

(*B. licheniformis*)

(*B. pumilus*)

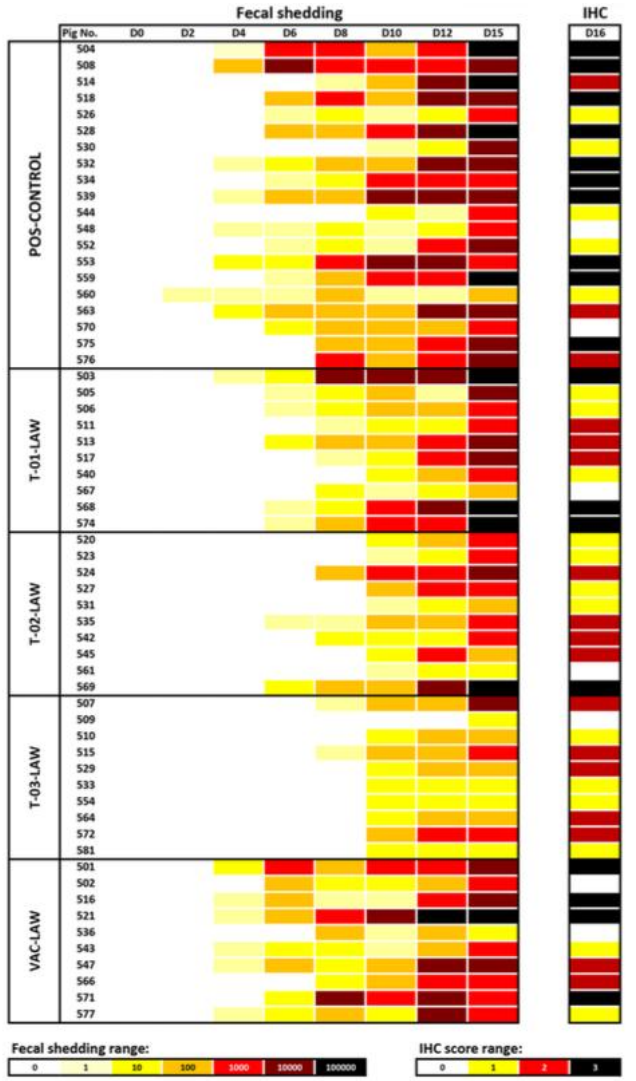


Figure 3 Group mean log10 L. intracellularis DNA in rectal swabs over time. Different superscripts (A,B,C) at a day post-challenge indicate a significant ($P < 0.05$) difference among groups. 图 3 随时间变化的直肠拭子胞内劳森菌 DNA 的各组平均 log10。挑战后一天的不同上标（A,B,C）表示组间差异显著（$P < 0.05$）。

UFMG

Vaccines 疫苗

- Vaccine (Enterisol® Ileitis, Boehringer Ingelheim) 疫苗（Enterisol® Ileitis，勃林格殷格翰公司）
- Vaccine (Porcilis Ileitis, Merck Animal Health) 疫苗（Porcilis Ileitis，默克动物保健公司）
- Other alternatives 其他替代品

veterinary microbiology

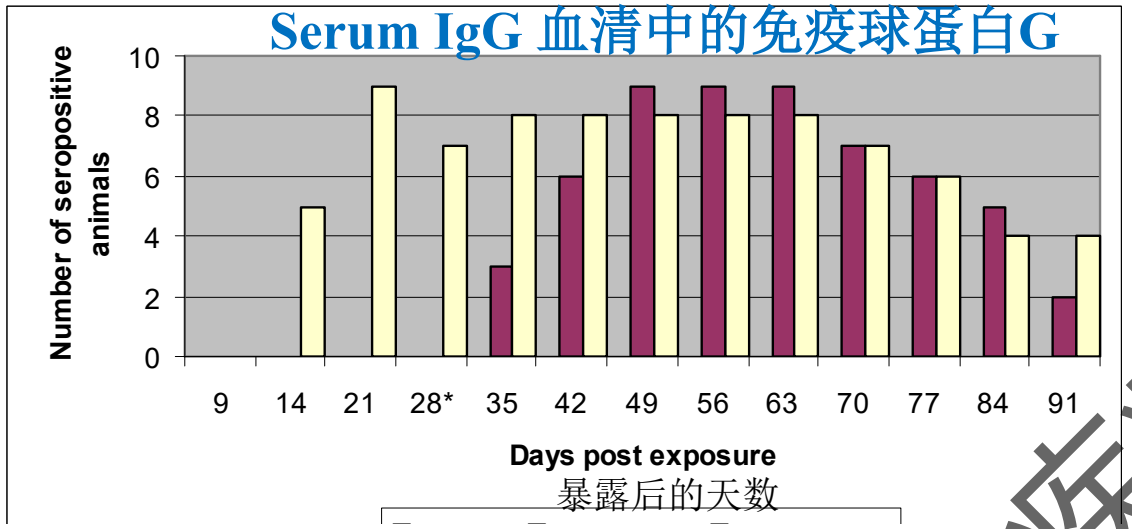
Onset and duration of fecal shedding, cell-mediated and humoral immune responses in pigs after challenge with a pathogenic isolate or attenuated vaccine strain of *Lawsonia intracellularis*

猪在接触致病性分离株或细胞内劳森氏菌减毒疫苗株后发生粪便脱落、细胞介导免疫反应和体液免疫反应的起始时间和持续时间

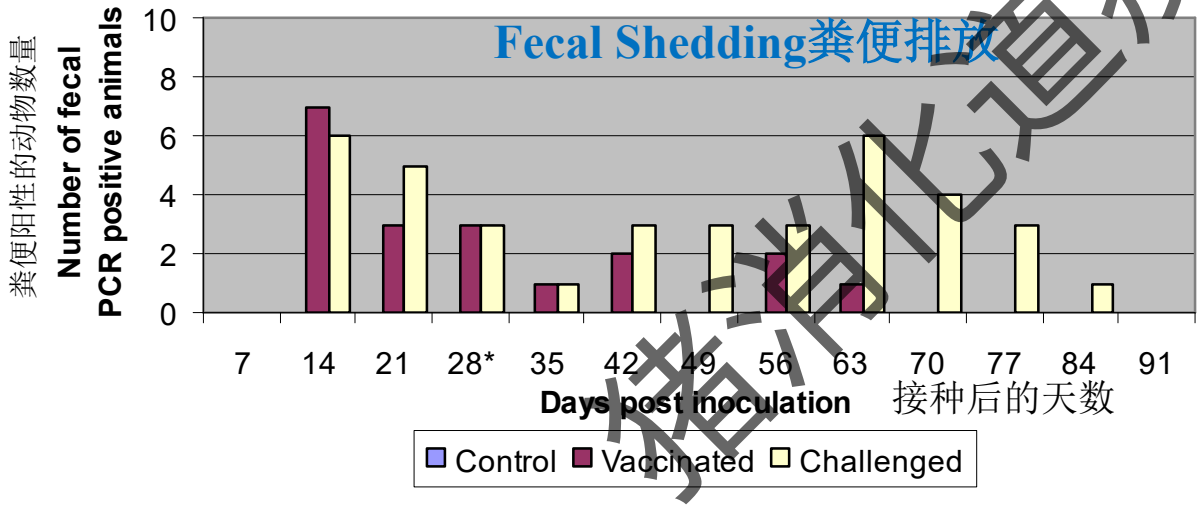
Roberto M.C. Guedes*, Connie J. Gebhart

罗伯托-毛里西奥-卡瓦略-格德斯

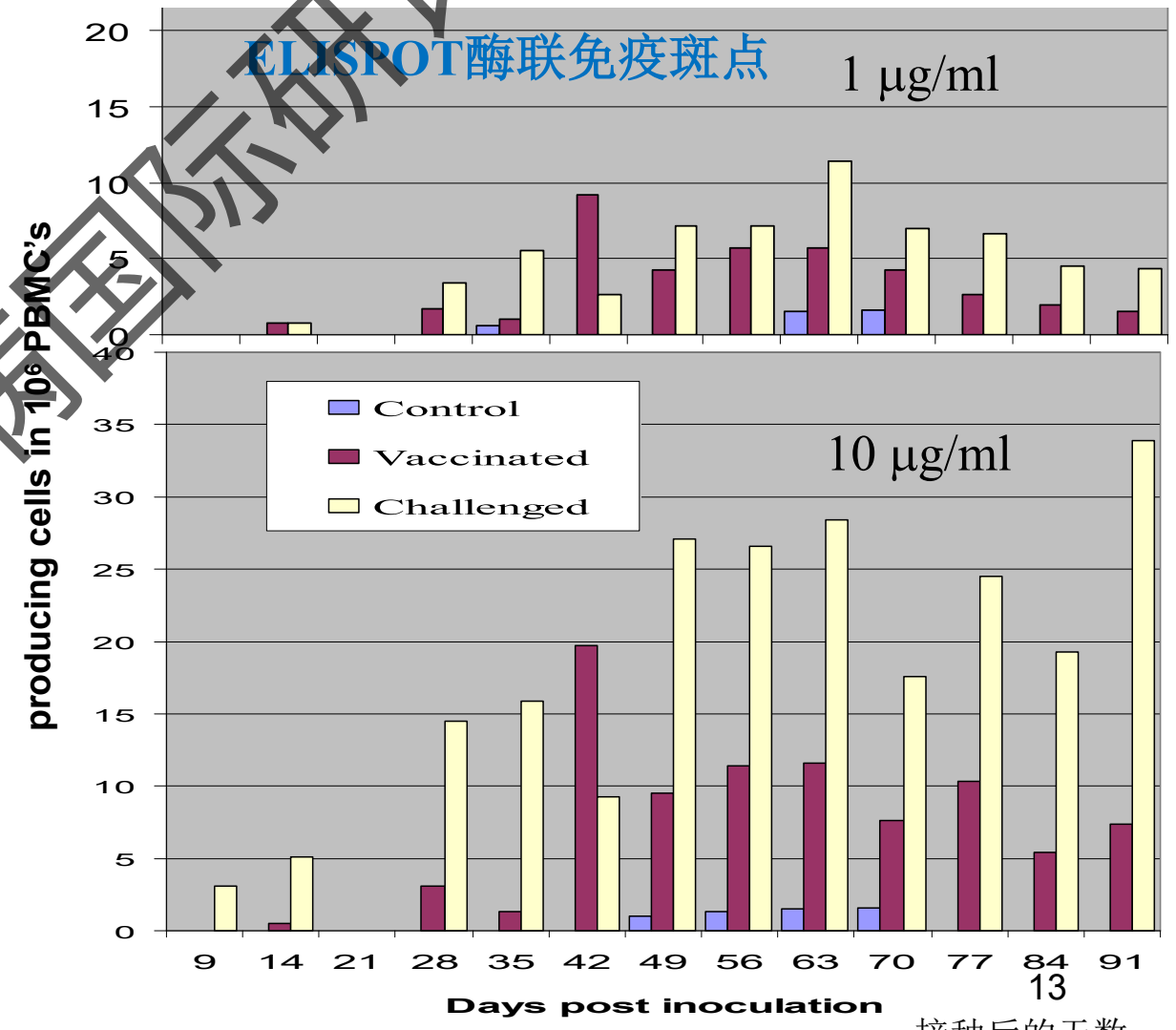
Serum IgG 血清中的免疫球蛋白G



Fecal Shedding粪便排放



ELISPOT酶联免疫斑点



Veterinary Microbiology 91 (2003) 135–145

Evaluation of protective immunity in pigs following oral administration of an avirulent live vaccine of Lawsonia intracellularis

Jeremy J. Kroll, BS; Michael B. Roof, PhD; Steven McOrist, BVSc, PhD

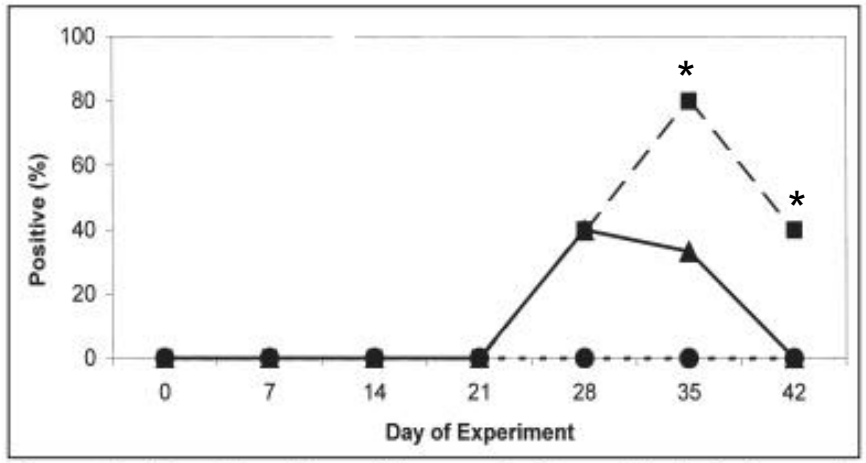


Figure 1—Fecal shedding of Lawsonia intracellularis in pigs orally vaccinated with an avirulent live vaccine and unvaccinated challenge-control pigs that were challenge exposed 21 days later with virulent L intracellularis during experiment 1. Fecal shedding was determined on the basis of positive results for a polymerase chain reaction assay. Fifteen pigs were vaccinated and challenged exposed (triangle), 10 pigs were unvaccinated and challenge exposed (square), and 10 pigs were unvaccinated and not challenge exposed (cirle). Day 0 is the day of oral administration of vaccine or culture medium.

Table 1—Mean ±SE scores for macroscopic and microscopic lesions in samples of ileum and colon obtained from 3 groups*of pigs in experiment 1

		Macroscopiclesionst		Microscopiclesionst	
Group		Ileum	Colon	Ileum	Colon
Vaccinates	15	1.5±0.19[a]	1.0±0.00[a]	0.4±0.19[a]	0.0±0.00[a]
Challenge-control pigs	10	3.6±0.22[b]	2.0±0.39[b]	2.4±0.34[b]	1.5±0.43[b]
Negative-control pigs	10	1.1±0.10[a]	1.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]

*Groups consisted of pigs vaccinated by oral administration of an avirulent live vaccine of Lawsonia intra-cellularis on day 0 and challenge exposed on day 21 with virulent heterologous L intracellularis (vaccinates), pigs that were orally administered culture medium on day 0 and challenge exposed on day 21 with virulent L intracellularis (challenge-control pigs), and pigs that were orally administered culture medium on day 0 and were not challenge exposed (negative-control pigs). tMacroscopic lesions were scored on a scale from 1 (normal) to 4 (severe thickening). tMicroscopic lesions were scored on a scale from 0 (normal) to 3 (severe-diffuse).
“Within a column, values with different superscript letters differ significantly (P< 0.05).

AJVR, Vol 65, No. 5, May 2004

Evaluation of protective immunity in pigs following oral administration of an avirulent live vaccine of Lawsonia intracellularis

评估口服胞内劳森菌无毒活疫苗后猪的保护性免疫力

Jeremy J. Kroll, BS; Michael B. Roof, PhD; Steven McOrist, BVSc, PhD

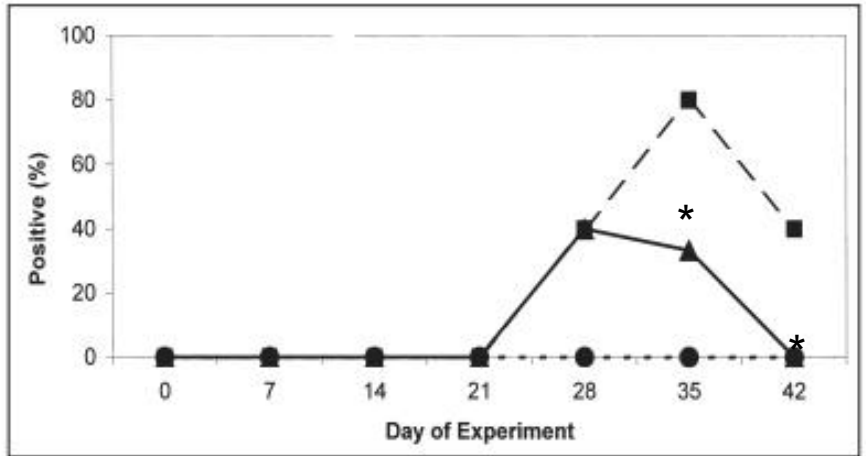


Figure 1—Fecal shedding of Lawsonia intracellularis in pigs orally vaccinated with an avirulent live vaccine and unvaccinated challenge-control pigs that were challenge exposed 21 days later with virulent L intracellularis during experiment 1. Fecal shedding was determined on the basis of positive results for a polymerase chain reaction assay. Fifteen pigs were vaccinated and challenged exposed (triangle), 10 pigs were unvaccinated and challenge exposed (square), and 10 pigs were unvaccinated and not challenge exposed (cirle). Day 0 is the day of oral administration of vaccine or culture medium.

图 1-实验 1 期间，口服无毒活疫苗的猪和未接种疫苗的挑战对照组猪的粪便中细胞内罗森菌的脱落情况，21 天后，这些猪受到细胞内罗森菌毒力的挑战。根据聚合酶链反应测定的阳性结果确定粪便脱落情况。15 头猪接种了疫苗并接触了病毒（三角形），10 头猪未接种疫苗并接触了病毒（方形），10 头猪未接种疫苗但未接触病毒（圆形）。第 0 天为口服疫苗或培养基的日期。

Table 2—Mean ± SE scores for microscopic lesions and percentage of tissues with positive results for immunohistochemical (IHC) analysis by use of Latvsonia-Specific monoclonal antibodies in sam-ples of ileum and colon obtained from 4 groups* of pigs in experiment 4 组猪的回肠和结肠样本中显微病变的中位数 ± SE 评分和使用拉氏菌特异性单克隆抗体进行免疫组化（IHC）分析结果呈阳性的组织百分比

	Ileum 回肠		Colon 结肠	
Group 分组	**Lesion scoret**病变评分	**IHC (%)免疫组化**	**Lesion scoret**病变评分	**IHC (%)免疫组化**
Vaccinates-Water delivery 饮水免疫	200.30 ± 0.18[a]	15[a]	0.15 ±0.11[a]	10[a,b]
Vaccinates-Jrench 灌服免疫	200.65 ± 0.25[a]	30[a]	0.25 ± 0.18[a,b]	10[a,b]
Challenge-control pigs 挑战对照组	192.32 ± 0.29[b] ‡	79[b]	0.70 ± 0.29[b]	35[b]
Strict-Control pigs 严格对照组	130.0 ± 0.00[c]	0†	0.0 ± 0.00[a]	0†

Groups consisted of pigs vaccinated by oral administration of an avirulent live vaccine of L intracellularis on day 0 via addition to the water supply and challenge exposed on day 21 with virulent heterologous L intracellularis (vaccinates-water delivery), pigs vaccinated by direct oral drench administration of a 2-mL volume to each pig and challenge exposed on day 21 with virulent L /ntrace/Zu/ar/s (vaccinates-drench), pigs admin istered culture medium on day 0 and challenge exposed on day 21 with virulent L intracellularis (challengecontrol pigs), and pigs that were not administered vaccine or culture medium and were not challenge exposed (strict-control pigs). tMicroscopic lesions were scored on a scale from 0 (normal) to 3 (severe-diffuse). tOne ileum sample had inflammation and fibrosis indicative of proliferative enteropathy and as a resuh, mucosal crypts had been shed and could not be evaluated. **Within a column, values with different superscript letters differ significantly (P< 0.05). * 组别包括在第 0 天通过在供水中添加细胞内 L 病毒无毒活疫苗进行口服接种，并在第 21 天接触带毒异源细胞内 L 病毒的猪（接种-供水）、 每头猪直接口服 2 毫升量的淋洗疫苗，并在第 21 天接触带毒 L /ntrace/Zu/ar/s （疫苗-淋洗）；第 0 天注射培养基，并在第 21 天接触带毒 L intracellularis（挑战对照猪）；未注射疫苗或培养基且未接触挑战的猪（严格对照猪）。有一个回肠样本出现了炎症和纤维化，表明出现了增生性肠病，因此粘膜隐窝脱落，无法进行评估。**一栏中，上标字母不同的数值差异显著（P< 0.05）。

†Group not included in the statistical analysis of percentage IHC positive samples.

未纳入 IHC 阳性样本百分比统计分析的组别。

AJVR, Vol 65, No. 5, May 2004

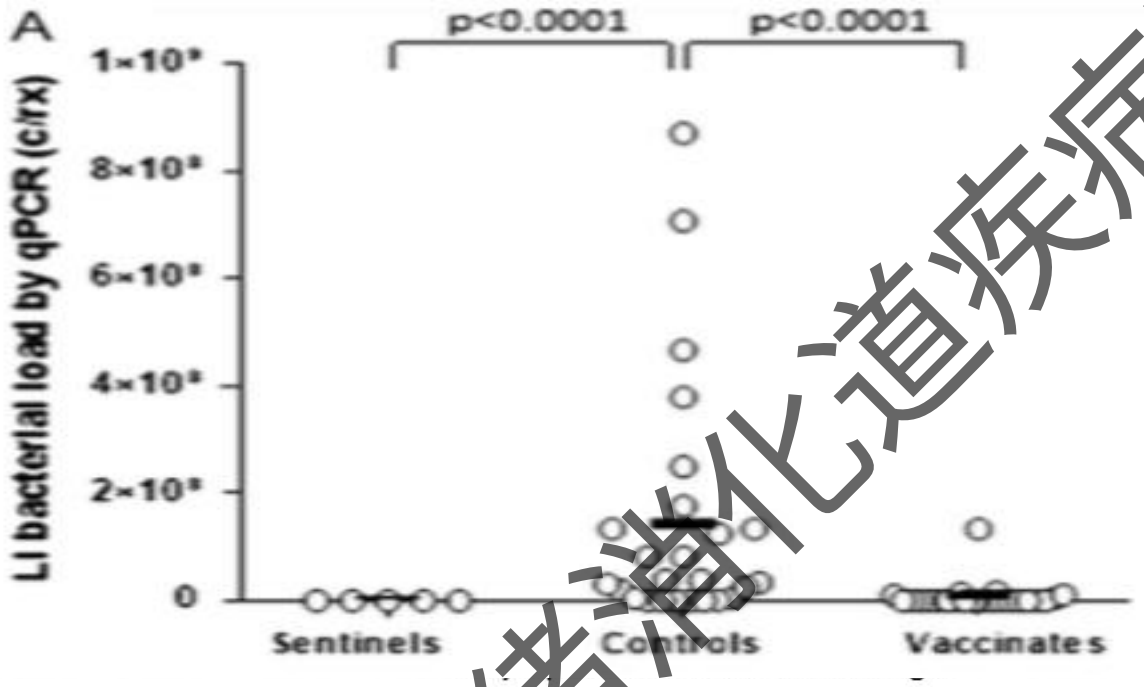
A novel inactivated vaccine against *Lawsonia intracellularis* induces rapid induction of humoral immunity, reduction of bacterial shedding and provides robust gut barrier function

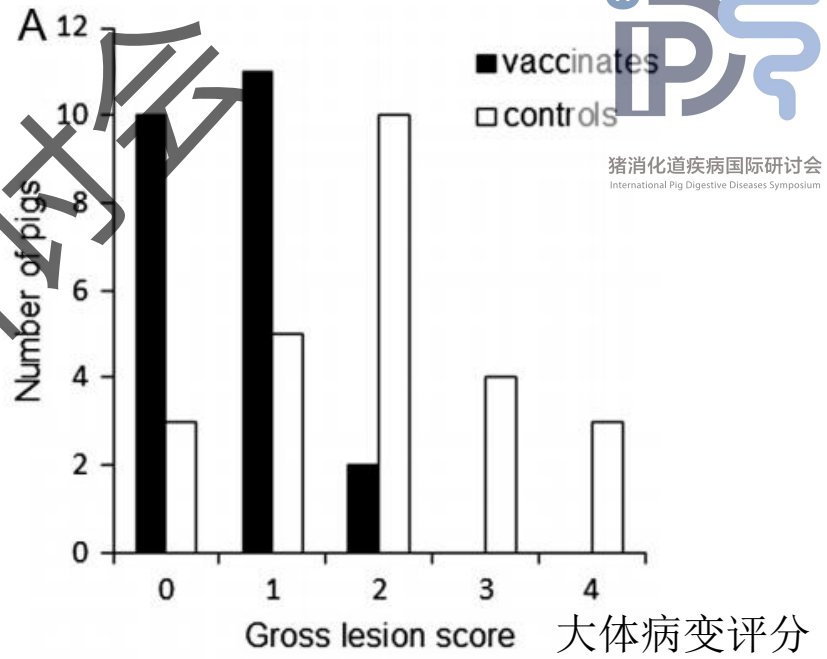
F. Roerink [a,*], C.L. Morgan [a], S.M. Knetter [a], M.-H. Passat [b], A.L. Archibald [b], T. Ait-Ali [b,*], E.L. Strait [a]

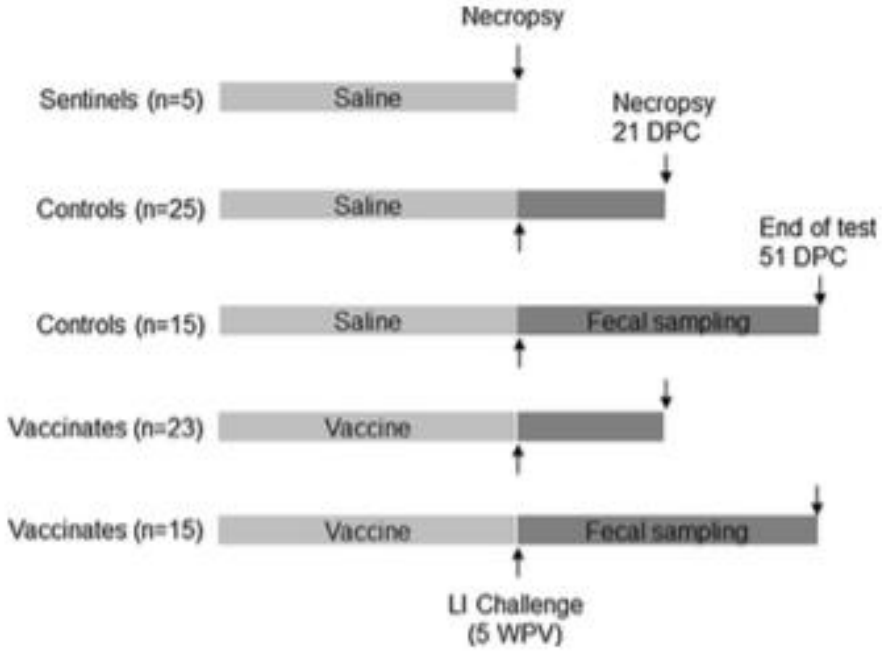
[a] Merck Animal Health, 2 Giralda Farms, Madison, NJ 07940, USA
[b] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Roslin, Midlothian EH25 9RG, UK

Vaccine 36 (2018) 1500–1508

一种新型细胞内劳森菌灭活疫苗可快速诱导体液免疫、减少细菌脱落并提供强大的肠道屏障功能



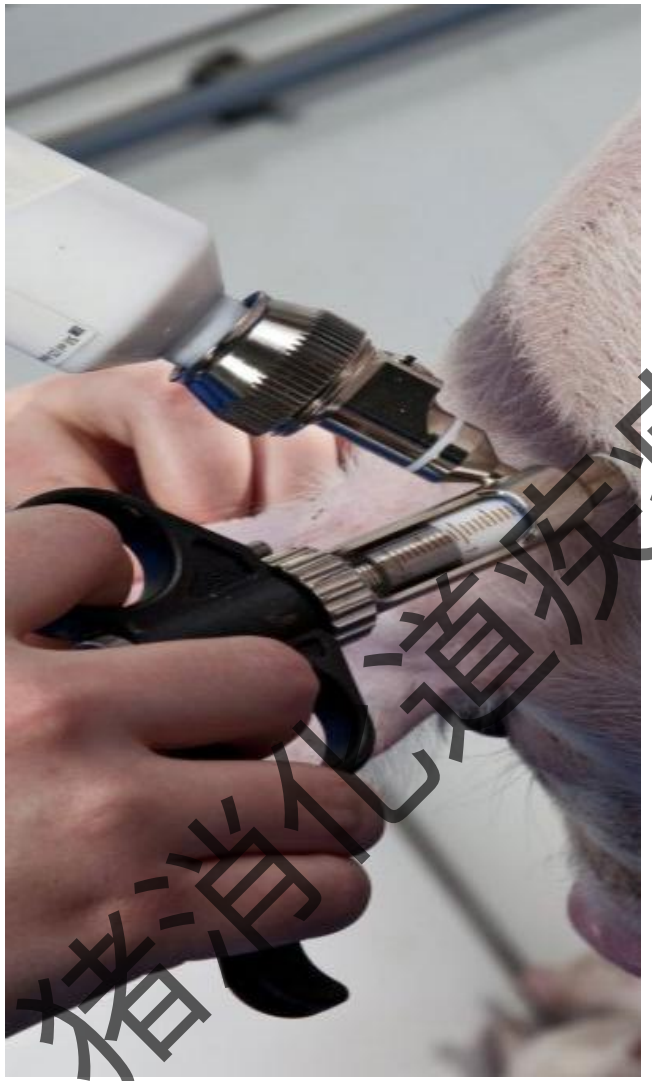




UFMG

What is new about *L. intracellularis* vaccines?

胞内劳森菌疫苗的新进展



失强效 O 抗原（粗糙型）的鼠伤寒沙门氏菌突变体，可分析与胞内劳森菌相关的抗原，增强了免疫原性，并在小鼠模型中为增生性肠炎和沙门氏菌病提供单次免疫保护

Potent O-antigen-deficient (rough) mutants of *Salmonella* Typhimurium secreting *Lawsonia intracellularis* antigens enhance immunogenicity and provide single-immunization protection against proliferative enteropathy and salmonellosis in a murine model

Suyeon Park[1†], Gayeon Won[1†], Jehyoung Kim[1], Hyeun Bum Kim[2*] and John Hwa Lee[1*]

VETERINARY RESEARCH

- LatA (OptA,OptB) Watson et al, 2011, LfliC Won & Lee, 2018, and Lhly Kim et al, 2017

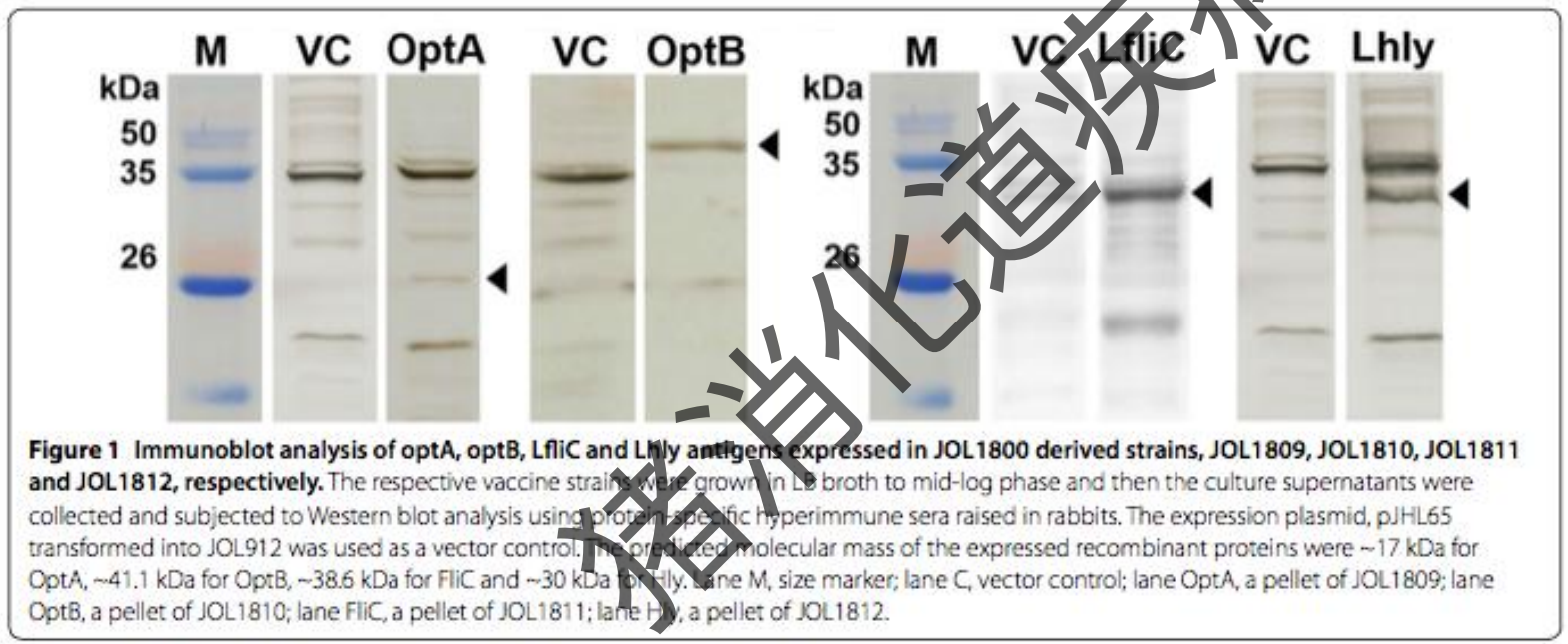


Figure 1 Immunoblot analysis of optA, optB, LfliC and Lhly antigens expressed in JOL1800 derived strains, JOL1809, JOL1810, JOL1811 and JOL1812, respectively. The respective vaccine strains were grown in LB broth to mid-log phase and then the culture supernatants were collected and subjected to Western blot analysis using protein-specific hyperimmune sera raised in rabbits. The expression plasmid, pJHL65 transformed into JOL912 was used as a vector control. The predicted molecular mass of the expressed recombinant proteins were ~17 kDa for OptA, ~41.1 kDa for OptB, ~38.6 kDa for FliC and ~30 kDa for Hly. Lane M, size marker; lane C, vector control; lane OptA, a pellet of JOL1809; lane OptB, a pellet of JOL1810; lane FliC, a pellet of JOL1811; lane Hly, a pellet of JOL1812.

- **实验方法：**使用LB培养基培养相应的疫苗菌株至对数生长期中期。收集培养上清液并进行Western blot分析。使用在兔子体内产生的特异性高免疫血清进行蛋白质特异性检测。
- **实验对照：**使用转化了表达质粒pJHL65的JOL912作为载体对照。
- **预期结果：**表达的重组蛋白的预期分子质量分别为：OptA约为17 kDa，OptB约为41.1 kDa，FliC约为38.6 kDa，Hly约为30 kDa。

Park et al. Vet Res (2018) 49:57
https://doi.org/10.1186/s13567-018-0552-8

Humoral imune respons体液免疫

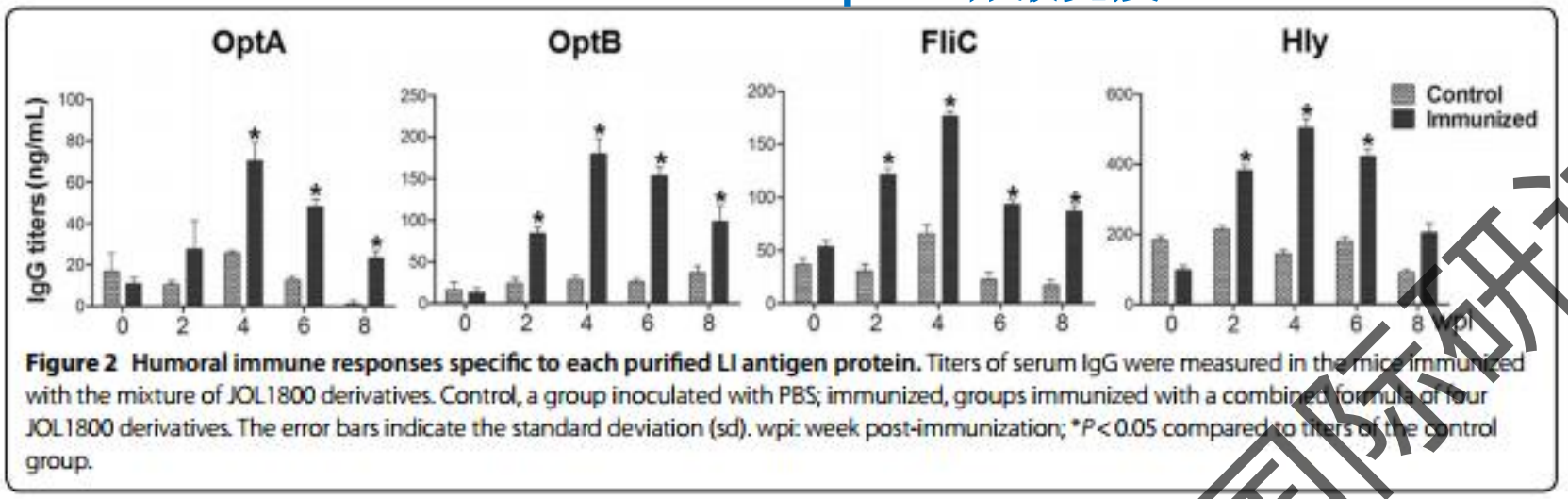


Figure 2 Humoral immune responses specific to each purified LI antigen protein. Titers of serum IgG were measured in the mice immunized with the mixture of JOL1800 derivatives. Control, a group inoculated with PBS; immunized, groups immunized with a combined formula of four JOL1800 derivatives. The error bars indicate the standard deviation (sd). wpi: week post-immunization; *$P < 0.05$ compared to titers of the control group.

. 5 wk-old Balb/c: 5周龄的Balb/c小鼠

Vac – oral cocktail of the 4 antigens
口服四种抗原的混合剂
Con – PBS 磷酸盐缓冲液

Serum, vaginal and intestinal washes collected at 0, 2, 4, 6 and 8 wks pv 在免疫前（pv）的0、2、4、6和8周收集血清、阴道和肠道冲洗液。

Mucosal imune response (intestinal and vaginal)
粘膜免疫反应（肠道和阴道）

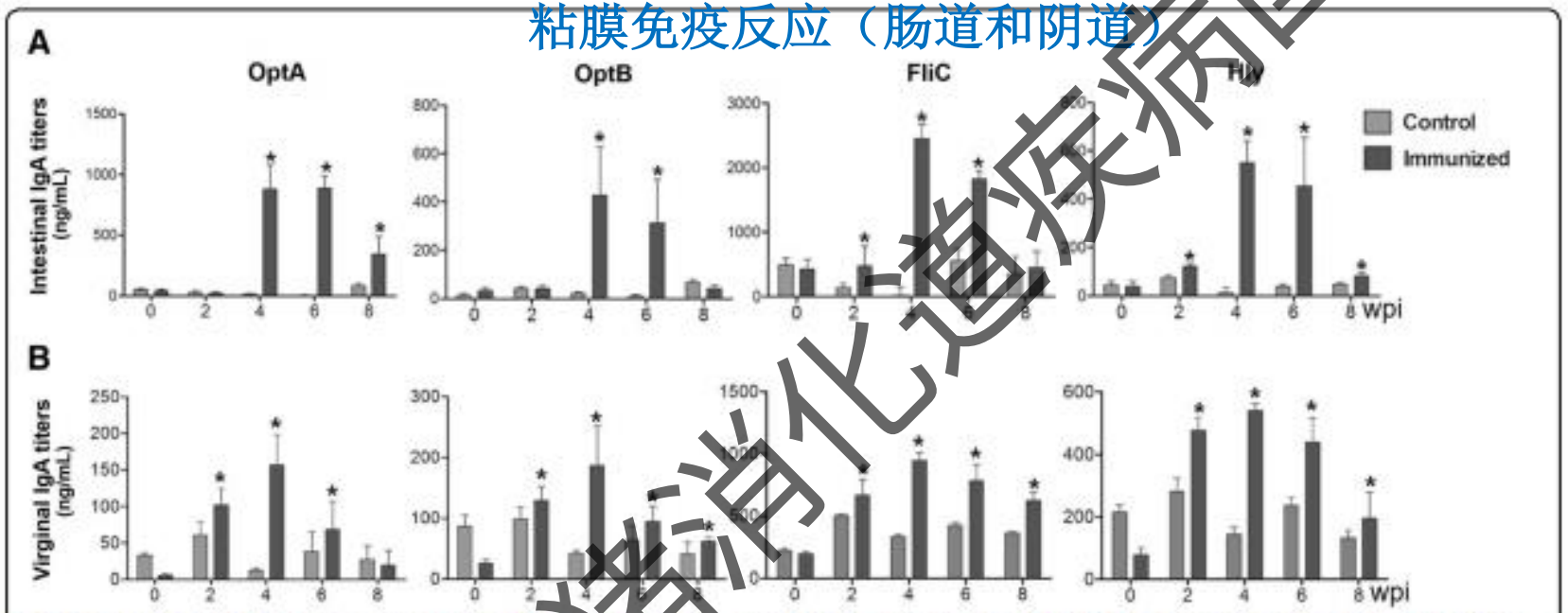


Figure 3 Mucosal immune response. Titers of intestinal secretory IgA (sIgA) **(A)** and virginal sIgA **(B)** obtained after vaccination with a co-mixture of all the four strains. The responses measured against each LI protein by an indirect ELISA using individual recombinant proteins as coating antigens. Each data points represent mean ± standard deviation (sd) of 10 mice/group. *$P < 0.05$. wpi: week post-immunization. ns: non-significant.

Park et al. Vet Res (2018) 49:57
https://doi.org/10.1186/s13567-018-0552-8

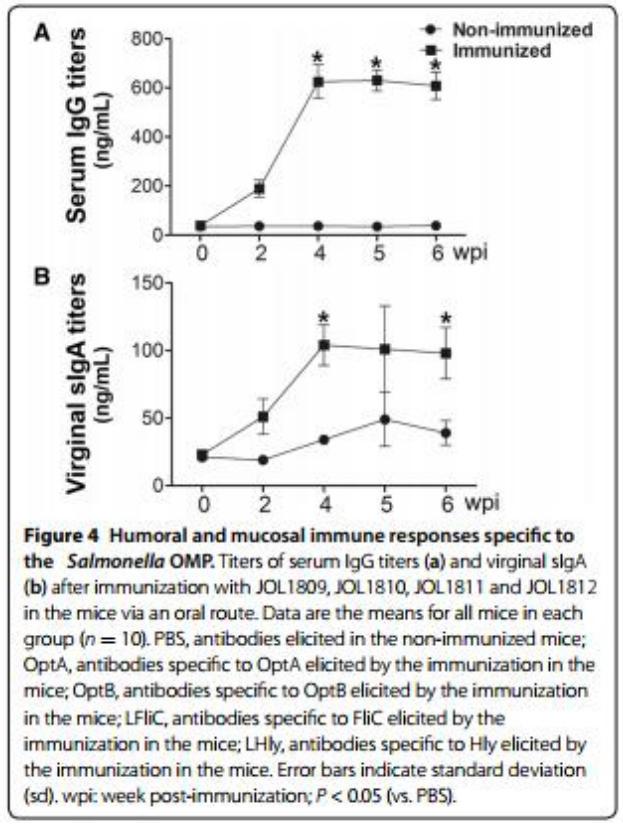


Figure 4 Humoral and mucosal immune responses specific to the *Salmonella* OMP. Titers of serum IgG titers (a) and virginal sIgA (b) after immunization with JOL1809, JOL1810, JOL1811 and JOL1812 in the mice via an oral route. Data are the means for all mice in each group ($n = 10$). PBS, antibodies elicited in the non-immunized mice; OptA, antibodies specific to OptA elicited by the immunization in the mice; OptB, antibodies specific to OptB elicited by the immunization in the mice; LFliC, antibodies specific to FliC elicited by the immunization in the mice; LHly, antibodies specific to Hly elicited by the immunization in the mice. Error bars indicate standard deviation (sd). wpi: week post-immunization; $P < 0.05$ (vs. PBS).

图4：小鼠通过口服途径接种不同菌株（JOL1809，JOL1810，JOL1811，和 JOL1812）后，针对沙门氏菌外膜蛋白（Salmonella OMP）的体液和粘膜免疫反应的实验结果。

免疫反应：实验测量了血清IgG（图 a）和阴道IgA（图 b）的抗体滴度，这两种抗体分别代表了体液和粘膜免疫反应。

数据表示：每个组别的数据是所有小鼠的平均值（n = 10），误差条表示标准偏差（sd）。

•抗体类型：PBS代表未免疫小鼠中产生的抗体；OptA、OptB、FliC、Hly分别代表特异性针对这些抗原的抗体，

•统计显著性：与PBS对照组相比，接种后特定时间点的抗体滴度有显著性差异（P < 0.05）

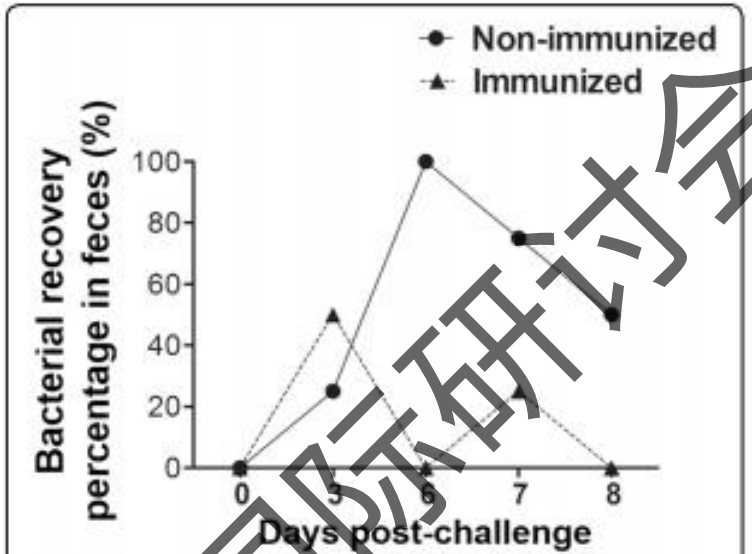


Figure 7 Protection efficacies of four rough ST-based LI vaccines. All the immunized and control mice groups were challenged with $10^{6.9}$ $TCID_{50}$ LI strains at week 8 post-immunization. Protection shown by a cocktail of all the four strains. The protection against LI was assessed based on the presence of gDNA in stool samples. Protection is presented as the number of animals showing gDNA in stool samples to the total number of animals in that group. Five animals were sacrificed at each time-point indicated.

5 wk-old C57BL/6: 5周龄的Balb/c小鼠
Vac – oral cocktail of the 4 antigens
口服四种抗原的混合剂
Con – PBS 磷酸盐缓冲液
Challenged 2 wks pv with modified-live LI vaccine and evaluate fecal shedding by PCR. 在接种疫苗后2周，小鼠通过口服方式给予改良活疫苗，通过PCR检测小鼠的粪便样本。

图6：基于四种粗糙型沙门氏菌的胞内劳森菌疫苗的保护效果
所有接种疫苗和对照组的小鼠在免疫后第8周接受了10^6.9个感染剂量的胞内劳森菌株的挑战。
实验结果：接种疫苗的小鼠组相比于未接种疫苗的对照组显示出更高的保护效果，即粪便中检测到gDNA的动物比例较低。

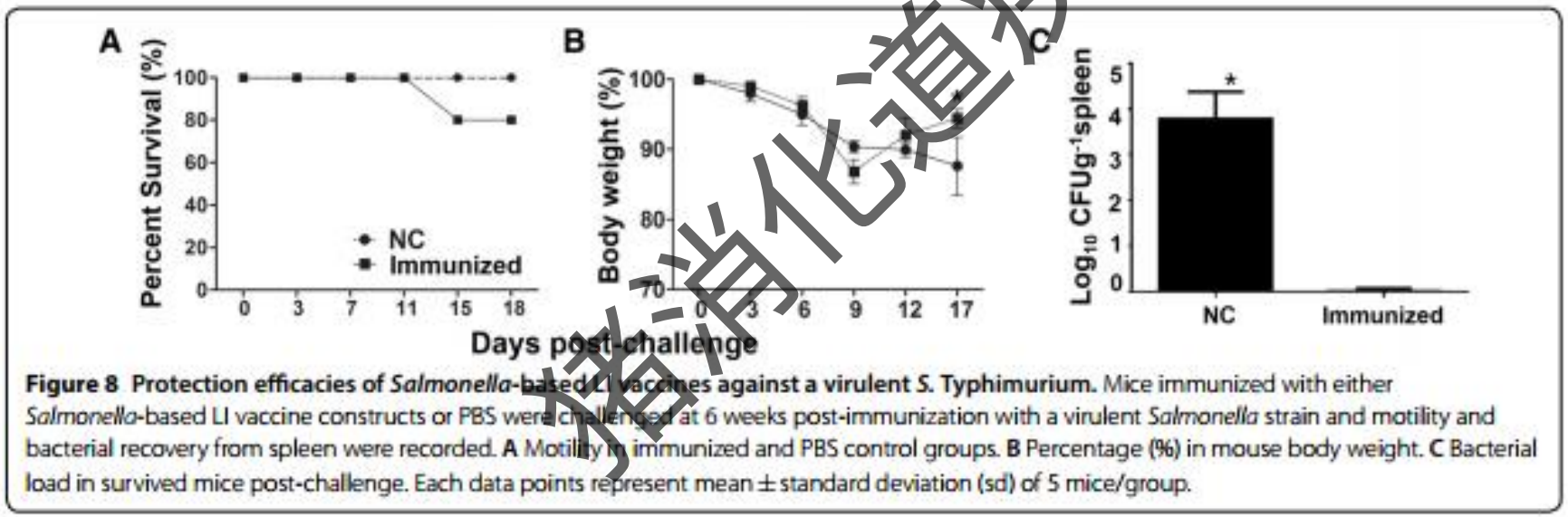


Figure 8 Protection efficacies of *Salmonella*-based LI vaccines against a virulent *S.* Typhimurium. Mice immunized with either *Salmonella*-based LI vaccine constructs or PBS were challenged at 6 weeks post-immunization with a virulent *Salmonella* strain and motility and bacterial recovery from spleen were recorded. **A** Motility in immunized and PBS control groups. **B** Percentage (%) in mouse body weight. **C** Bacterial load in survived mice post-challenge. Each data points represent mean ± standard deviation (sd) of 5 mice/group.

图8：小鼠在免疫后第6周接受了强毒力沙门氏菌菌株的挑战，以评估基于沙门氏菌的胞内劳森菌疫苗构建的保护效果。
评估指标：
- A. 免疫组和PBS对照组的小鼠运动性（Motility）。
- B. 小鼠体重百分比变化。
- C. 挑战后存活小鼠体内的细菌负荷（Bacterial load）。
- **数据表示**：每个数据点代表每组5只小鼠的平均值 ± 标准偏差（sd）。

Park et al. Vet Res (2018) 49:57
https://doi.org/10.1186/s13567-018-0552-8

Contents lists available at ScienceDirect

Vaccine

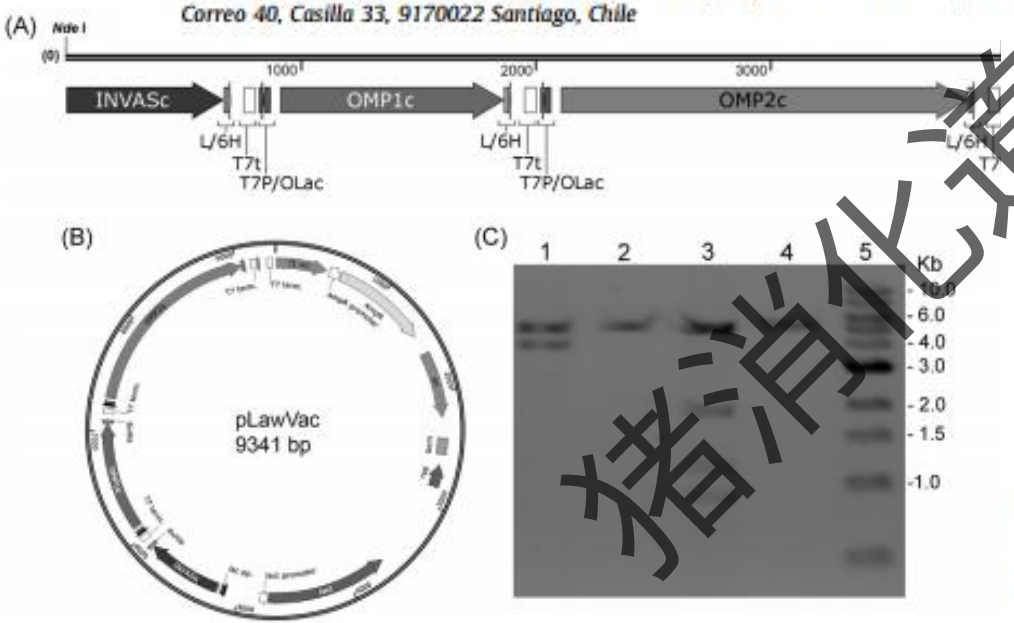
journal homepage: www.elsevier.com/locate/vaccine

针对猪回肠炎病原体胞内劳森菌的多抗原重组亚单位疫苗

Multi-antigenic recombinant subunit vaccine against *Lawsonia intracellularis*: The etiological agent of porcine proliferative enteropathy

R. Montesino [a,1], N. Gutiérrez [a], F. Camacho [b], O. Farnós [c], S. Andrades [d], A. González [a], J. Acosta [a], M. Cortez-San Martín [e], O. Sánchez [b], A Ruiz [d], J.R. Toledo [a,*]

[a] Biotechnology and Biopharmaceuticals Laboratory, Pathophysiology Department, School of Biological Sciences, Universidad de Concepción, Victor Lamas 1290, P.O. Box 160C, Concepción, Chile
[b] Pharmacology Department, School of Biological Sciences, Universidad de Concepción, Victor Lamas 1290, P.O. Box 160C, Concepción, Chile
[c] Department of Bioengineering, Faculty of Engineering, McGill University, 141 Avenue du Président-Kennedy, Montréal, Canada
[d] Pathology and Preventive Medicine Department, School of Veterinary Sciences, Universidad de Concepción, Avenida Vicente Méndez 595, Chillan, Chile
[e] Molecular Virology and Pathogen Control Laboratory, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile (USACH), Alameda 3363, Correo 40, Casilla 33, 9170022 Santiago, Chile



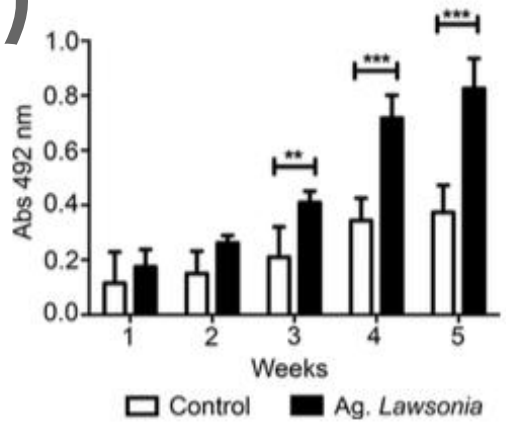


Fig. 4. Time-course of IgG response against *L. intracellularis* antigens in mice. Sera were evaluated by indirect ELISA. Bars represent the standard deviation of IgG values. Results were statistical analyzed by a Kruskal-Wallis test and the Dunn post-test of multiple comparisons (***$p \leq 0.001$, **$p \leq 0.01$).

图4：通过ELISA评估小鼠血清中针对胞内劳森菌抗原的IgG 水平随时间变化的情况
Kruskal-Wallis 测试进行结果的统计分析，并使用 Dunn 多重比较后检验来确定不同时间点间的差异是否显著
"'p ≤ 0.001" 表示差异极其显著，"p ≤ 0.01" 表示差异显著

图6：经过不同处理的猪对胞内劳森菌 IgG 抗体反应

- **实验组别**：包括对照组（Control）和接种了胞内劳森菌的疫苗以及额外添加了干扰素的组别。
- **统计分析**：通过双向方差分析（two-way ANOVA）和 Tukey 多重比较后检验来进行数据分析，以确定不同组别间的统计学差异。
- **显著性标记**：同图4

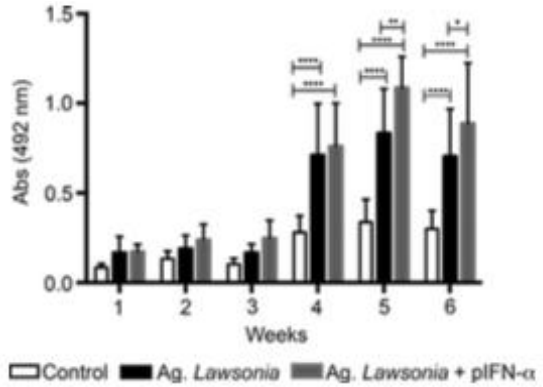


Fig. 6. Humoral immune response against *L. intracellularis* antigens assayed in pigs. The IgG antibody response was measured by indirect ELISA. Bars represent the standard deviation. The data statistical analysis was performed by a two way ANOVA and a Tukey's post-test of multiple comparisons (****$p \leq 0.0001$, **$p \leq 0.01$, *$p \leq 0.05$).

胞内劳森菌的免疫蛋白组学分析确定了亚单位疫苗开发中使用的候选中和抗体靶点

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Immunoproteomic analysis of *Lawsonia intracellularis* identifies candidate neutralizing antibody targets for use in subunit vaccine development

Milan Obradovic[a,b], J. Alex Pasternak[c], Siew Hon Ng[a], Brenda Allan[a], Robert Brownlie[a], Heather L. Wilson[a,b,*]

[a] Vaccine and Infectious Disease Organization (VIDO)-International Vaccine Centre (InterVac), University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5E3, Canada
[b] School of Public Health, Vaccinology and Immunotherapeutics, Saskatchewan, Canada
[c] Large Animal Clinical Sciences (LACS), Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5B4, Canada

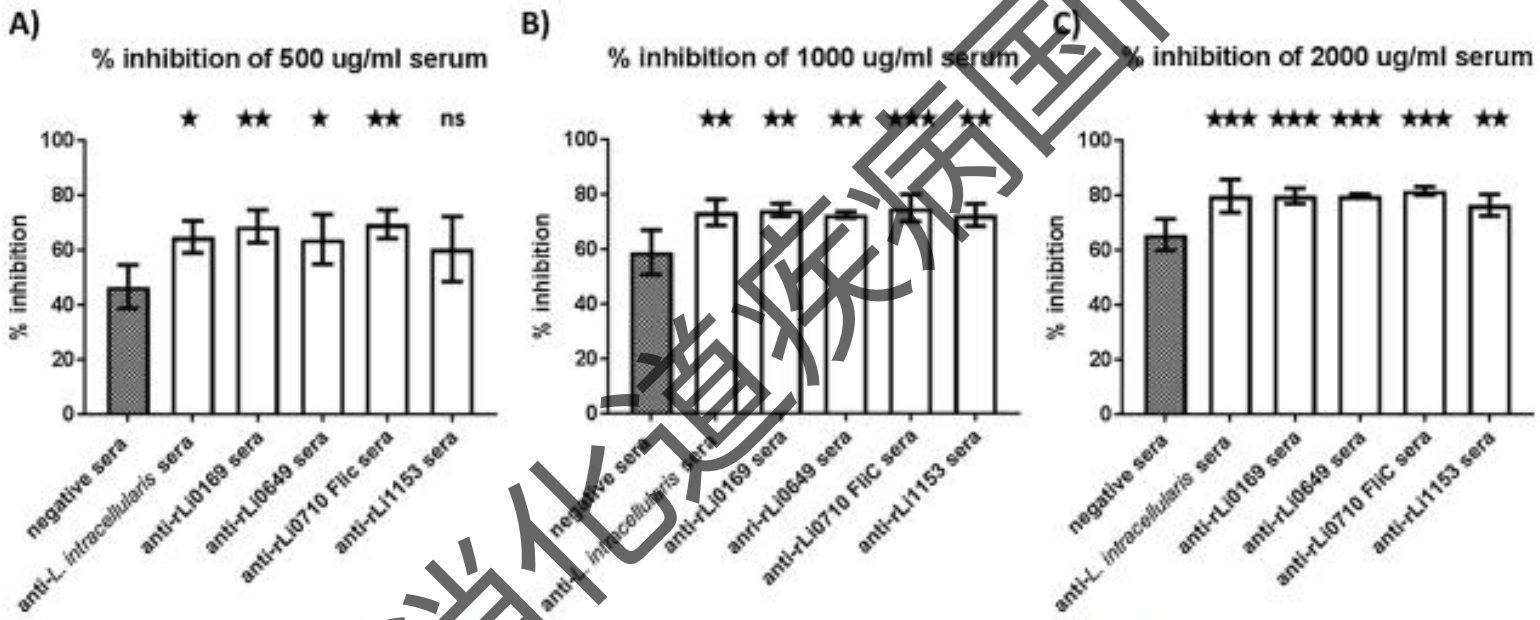


Fig. 4. Inhibitory effect of rabbit sera on CFSE- labelled avirulent L. intracellularis penetration in IPEC-1 cells: negative control sera (sera obtained prior to immunization, pooled and precleared of anti-LPS antibodies), anti-L. intracellularis sera (serum from rabbits immunized with whole avirulent bacteria), sera from rabbits immunized with recombinant proteins: anti-rLI0169, anti-rLI0649, anti-rLI0710 FliC, anti-rLI1153; serum concentrations used in assay 500 µg/ml (A); 1000 µg/ml (B) and 2000 µg/ml (C). All sera were cleared from antibodies against LPS. Percent inhibition = (1- % of fluorescence of CFSE bacteria incubated with serum / % of fluorescence of CFSE bacteria (control)) x 100. Data presented for 4 biological replicates. The error bar shows standard deviation of mean value of 4 biological replicates. ((***) $p < 0.001$, (**) $p < 0.01$ and (*) $p < 0.05$, (ns) not significant.

实验设计：实验使用了不同浓度的血清（500 µg/ml、1000 µg/ml 和 2000 µg/ml）来评估其对细菌侵入细胞的抑制作用。

血清类型：实验中使用了几种不同类型的血清，包括：

- 阴性对照血清（在免疫前获得的混合血清，预先清除了抗LPS抗体）。
- 抗 L. intracellularis 血清（来自接种了整个非致病性细菌的兔子的血清）。
- 接种了重组蛋白的兔子血清：包括抗 rLI0169、抗 rLI0649、抗 rL10710 FliC 和抗 rL11153。

实验结果：通过使用 CFSE 标记的 L. intracellularis 细菌和不同浓度的血清进行共培养，然后测量荧光强度来计算抑制百分比。实验结果显示了不同血清对细菌侵入细胞的抑制效果。

数据分析：实验数据以四个生物学重复的平均值和标准偏差呈现。使用误差条表示平均值的标准偏差。

显著性：实验结果中，(***) 表示 p 值小于 0.001，(**) 表示 p 值小于 0.01，(*) 表示 p 值小于 0.05，而 (ns) 表示结果不显著。

Biggest challenges for PPE control
控制回肠炎的最大挑战

- Lack of epidemiology data 缺少流行病学数据
- Lack of a field diagnostic test 缺乏现场诊断测试
- Difficult detection and evaluation of subclinical disease亚临床疾病的难以检测和评估
- Very few aditives as an alternative of control作为控制替代品的添加剂非常少
- Medication vs Vaccination? 药物治疗vs疫苗免疫？

Swine Pathology Group
猪病理团队

Ackwolegdments
致谢

Collaborations:合作的个人和机构
University of Minnesota – Prof. Connie Gebhart & Prof. Fabio Vannuccci明尼苏达大学
Chualalankor University – Prof. Suphot Wattanaphansak 朱拉隆功大学
Danish Technical University – Prof. Tim Jensen & Prof. Gregers Jungersen丹麦技术大学
The Roslin Institute/University of Edingburgh – Prof. Tahar Ait-Ali & Profa. Jill Thompson
罗斯林研究所 /爱丁堡大学等。