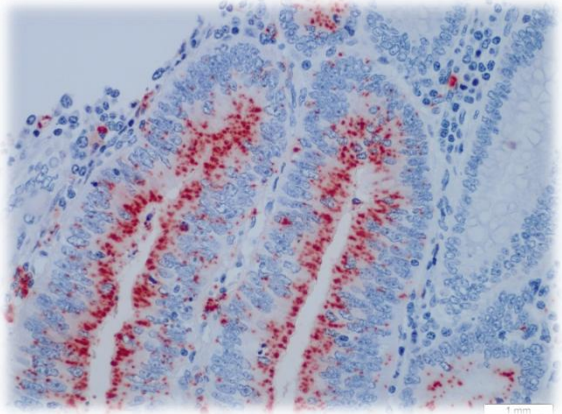
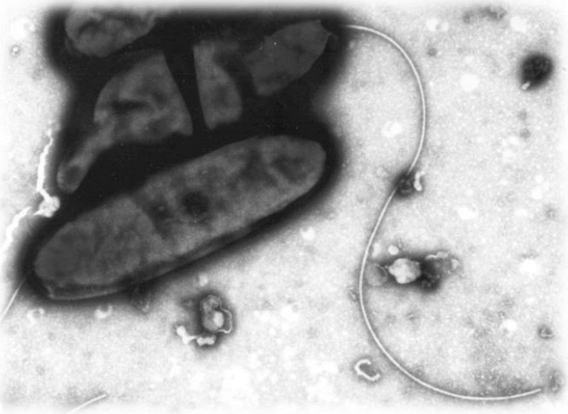
“Serology as a tool for understanding *Lawsonia intracellularis* kinetics of infection”
将血清学作为了解胞内劳森菌感染动态的工具

Roberto Guedes
罗伯托·格德斯

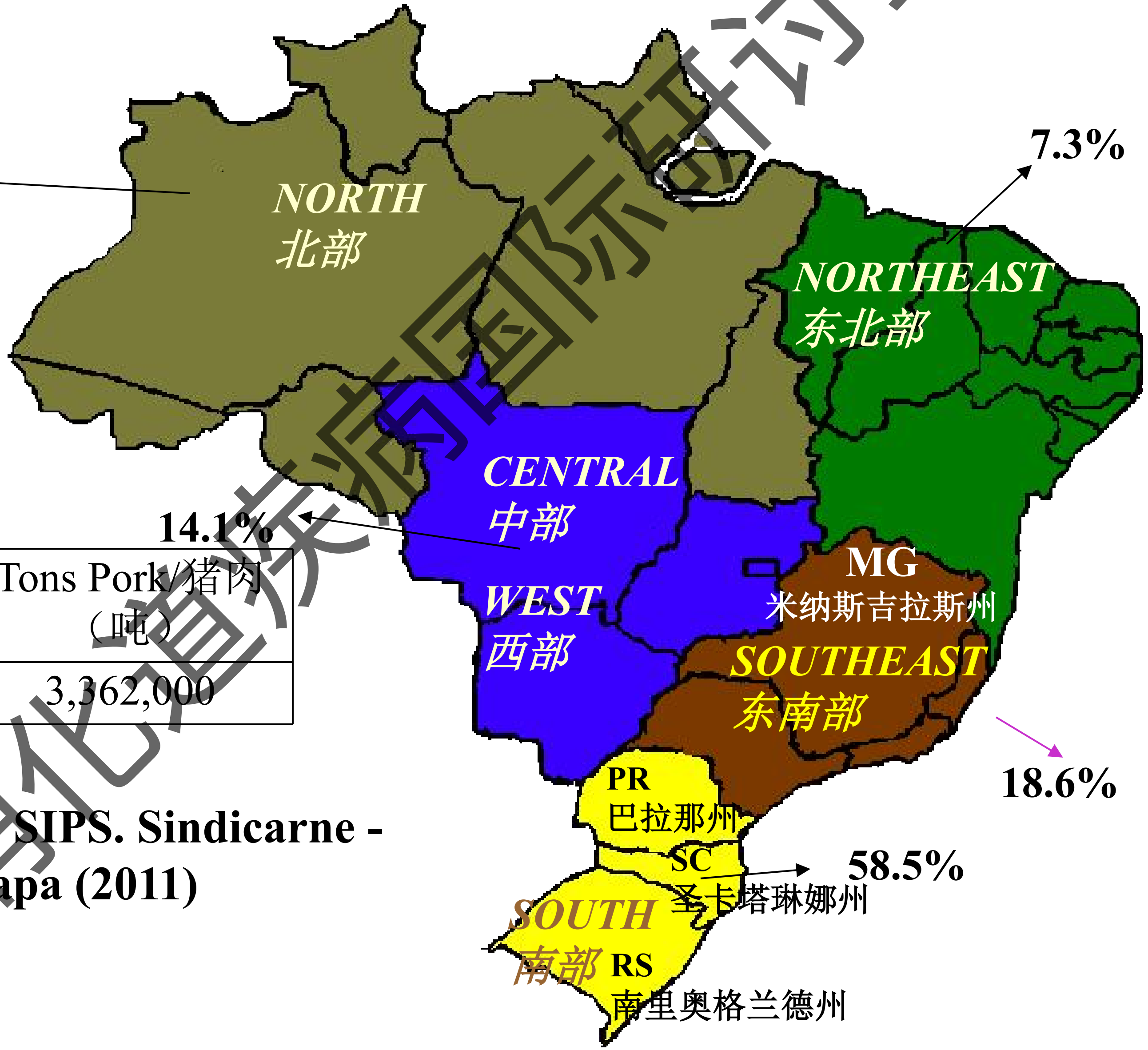
Universidade Federal de Minas Gerais
米纳斯吉拉斯联邦大学

邮箱： guedesufmg@gmail.com





SWINE PRODUCTION IN BRAZIL
巴西的养猪生产情况

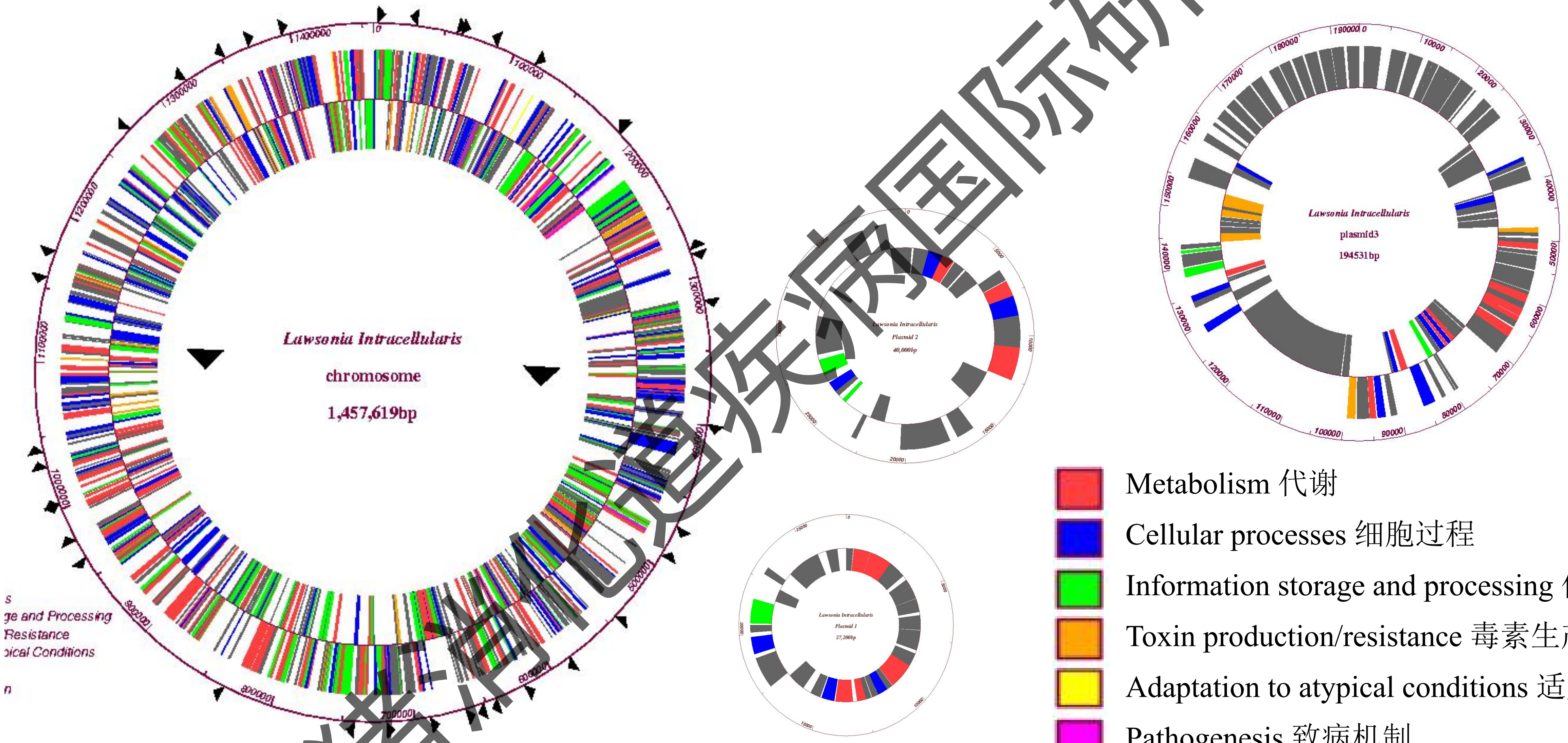


REGION/地区	Slaughtered pigs/屠宰猪	Tons Pork/猪肉（吨）
BRAZIL/巴西	30,807,512	3,362,000

SOURCE（数据来源）：**Abipecs, SIPS. Sindicarne - PR, Sindicarne- SC, ABCS, Embrapa (2011)**

Complete *L. intracellularis* Genome
(Gebhart & Kapur, 2003)
1,719,350 bp; 1,346 ORFs

完整的胞内劳森菌基因组
(Gebhart & Kapur, 2003)
1,719,350个碱基对；1,346个开放阅读框（ORF）



- Metabolism 代谢
- Cellular processes 细胞过程
- Information storage and processing 信息存储和处理
- Toxin production/resistance 毒素生产/耐药性
- Adaptation to atypical conditions 适应非典型条件
- Pathogenesis 致病机制
- Unknown function 未知功能

UFMG

MICROBIAL GENOMICS 2020（2020年）

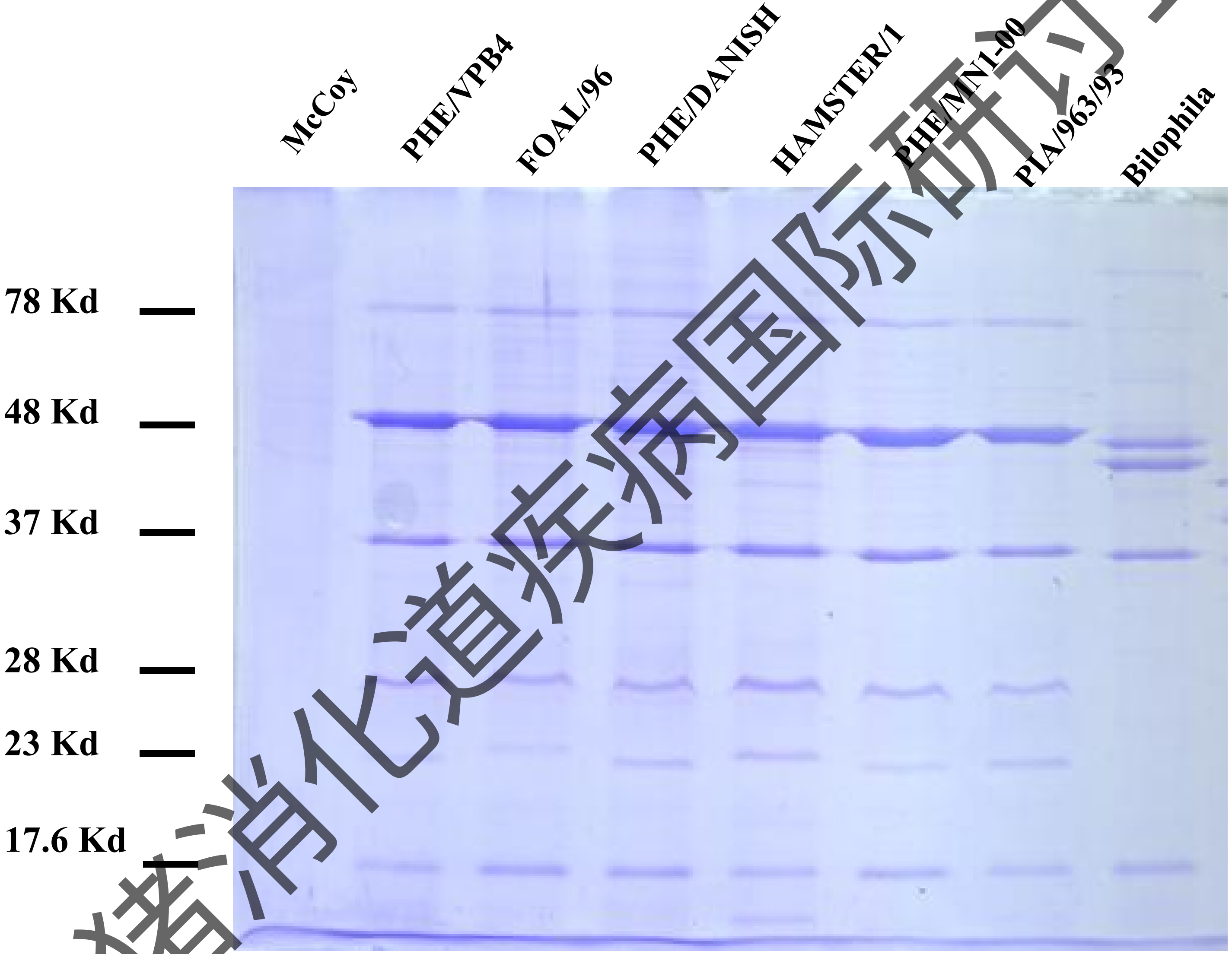
Metagenomic sequencing of clinical samples reveals a single widespread clone of *Lawsonia intracellularis* responsible for porcine proliferative enteropathy

Rebecca J. Bengtsson[1,2], Bryan A. Wee[1,3], Gonzalo Yebra[1], Rodrigo Bacigalupe[1,4], Eleanor Watson[5], Roberto M. C. Guedes[6], Magdalena Jacobson[7], Tomasz Stadejek[8,9], Alan L. Archibald[1], J. Ross Fitzgerald[1,*] and Tahar Ait-Ali[1,*]

临床样本的宏基因组测序显示，胞内劳森菌中广泛分布着一种会引发猪增生性回肠炎的单一克隆体

L. intracellularis strains differences ? 胞内劳森菌菌株之间有何不同？

Fenotypic features: Outer membrane protein profile 表型特征：外膜蛋白质谱



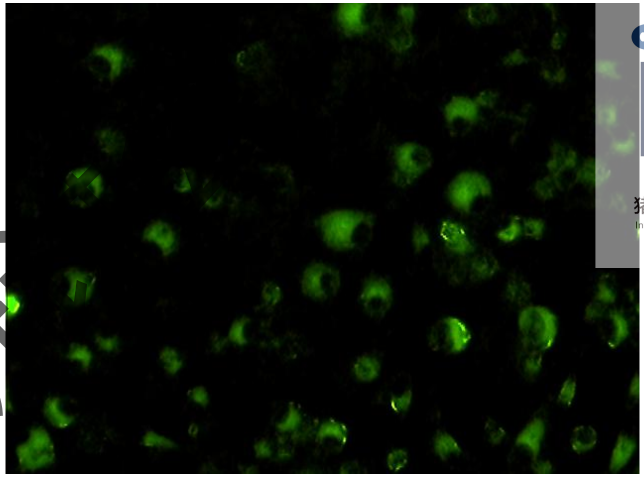
Serology (IgG) 血清学检测（IgG）

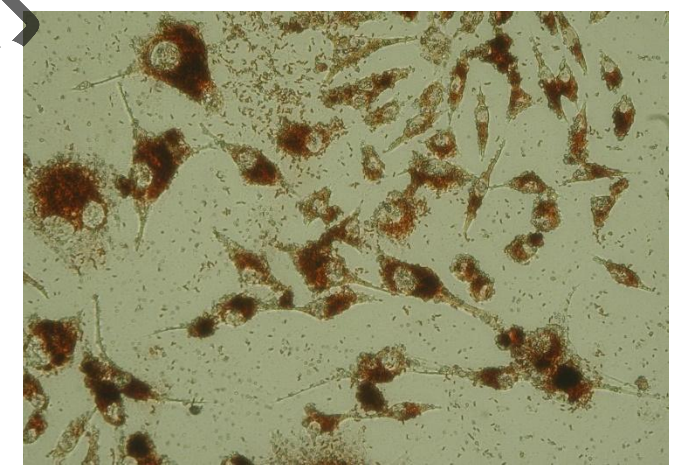
- IFA – plates (Knittel et al, 1998)
  免疫荧光检测（IFA）-平板（Knittel等，1998）
- IPMA – plates (Guedes et al, 2002 - USA)
  免疫过氧化物酶单层含量测定（IPMA）-平板（Guedes等，2002 - 美国）

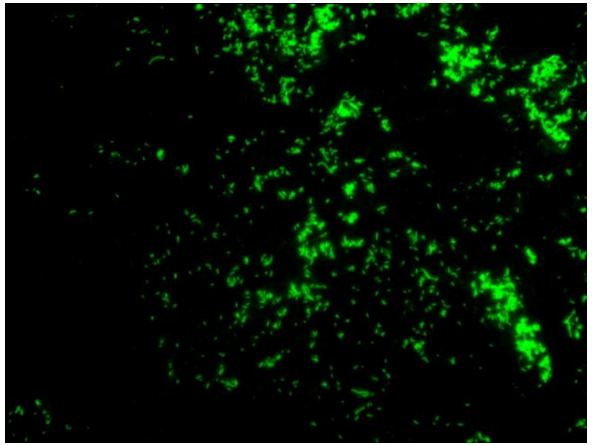
  Sens 89% Spec 100 %
  敏感度89%，特异性100%
- IFA – slides (Ileitest)
  IFA- 玻片（Ileitest）
- Elisa (Boesen et al, 2005 – Denmark)
  酶联免疫吸附测定ELISA（Boesen等，2005 - 丹麦）

  Sens 97% Spec 99.3%
  敏感度97%，特异性99.3%
- LPS Elisa (Kroll et al, 2005 – USA/Germany) Sens 88.7 % Spec 93.7 %
  脂多糖ELISA（Kroll等，2005 - 美国/德国）敏感度88.7%，特异性93.7%
- Blocking ELISA (Keller et al, 2006 – Svanovir *L. intracellularis*-Ileitis-Ab)
  阻断ELISA（Keller等，2006 - Svanovir L. intracellularis-Ileitis-Ab）
- Flow Cytometry Antibody Test – FCAT (Baldasso et al, 2023)
  流式细胞术抗体测试 - FCAT（Baldasso等，2023）

  Sens 98.8 % Spec 100%
  敏感度98.8%，特异性100%





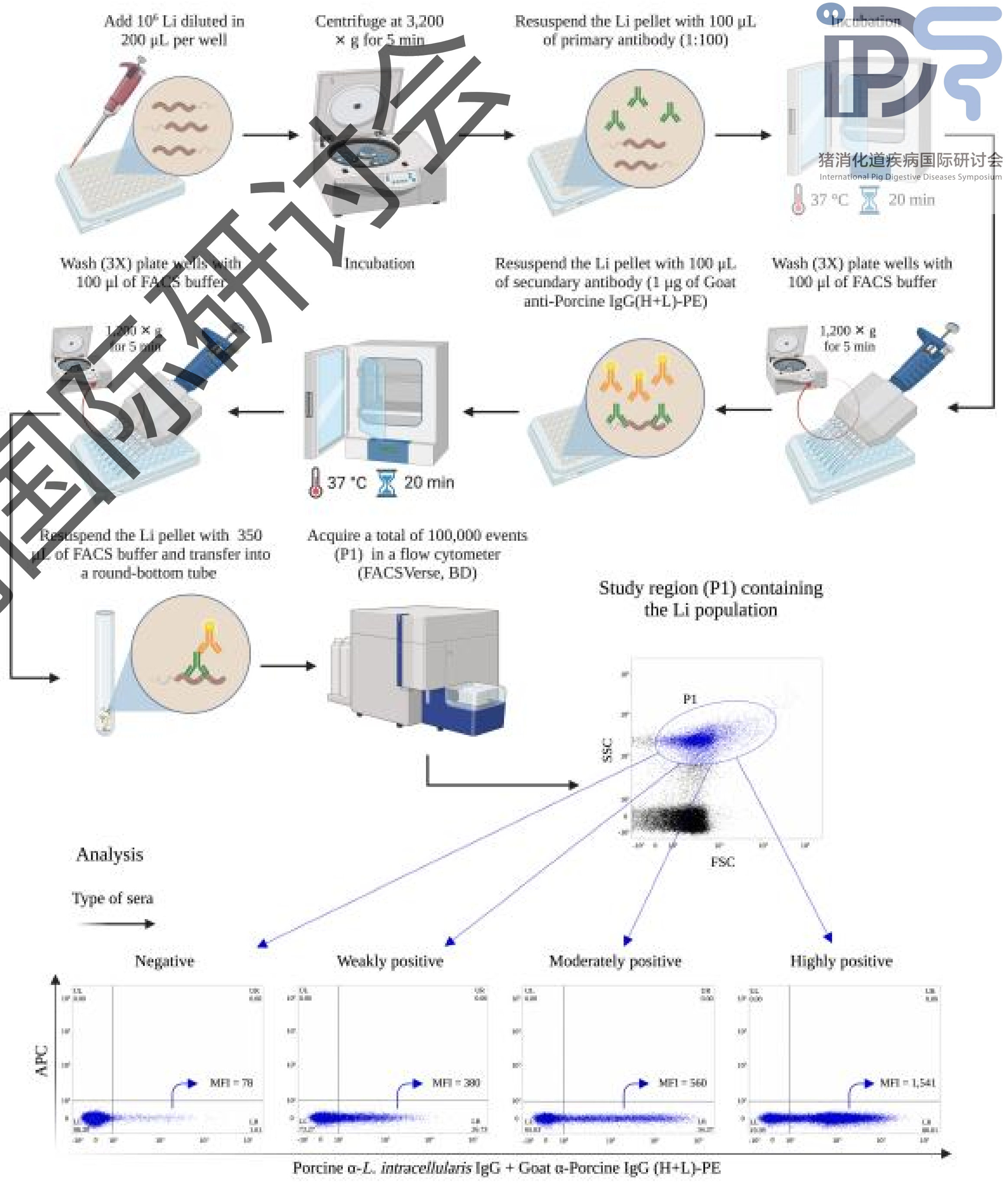


Development and validation of a flow cytometry antibody test for *Lawsonia intracellularis*

Frontiers in Immunology

Baldasso et al 2023
（Baldasso等，2023）

开发和验证用于胞内劳森菌的流式细胞术抗体测试



Development and validation of a flow cytometry antibody test for Lawsonia intracellularis
开发和验证用于胞内劳森菌的流式细胞术抗体测试

Frontiers in Immunology

Baldasso et al 2023
（Baldasso等，2023）

TABLE 4 Matrix used to calculate the Kappa index.
表4：用于计算Kappa指数的矩阵。

		FCAT		
		Positive 阳性	Negative 阴性	Total 合计
IPMA	Positive 阳性	26	10	36
	Negative 阴性	4	48	52
	Total 合计	30	58	88

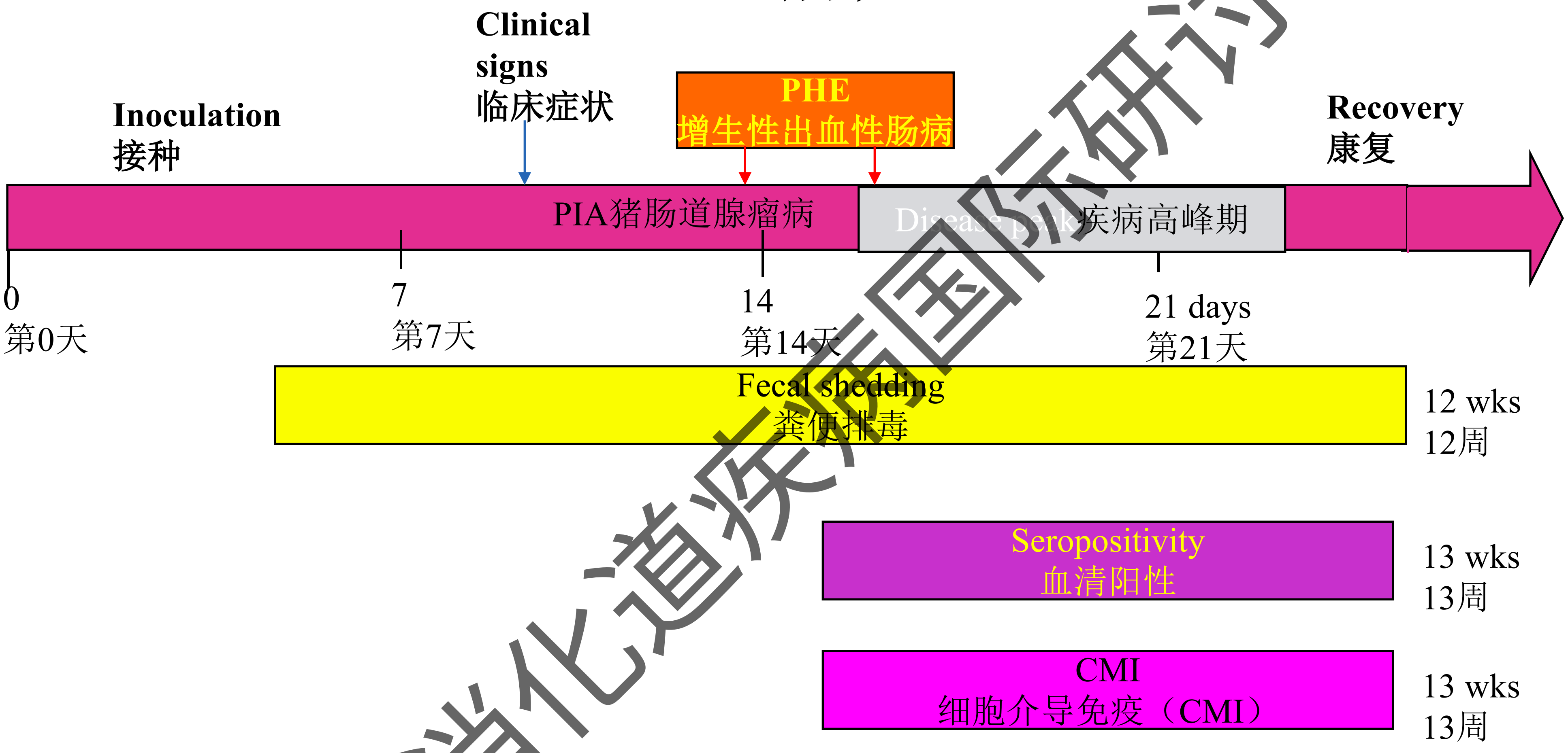
The calculation was performed online using the "Quantify Agreement with Kappa" tool from the GraphPad by Dotmatics website.
通过GraphPad by Dotmatics网站上的"Quantify Agreement with Kappa"工具进行计算。

TABLE 3 Serological evolution of pigs immunized or non-immunized pigs with *Lawsonia intracellularis*.
表3：胞内劳森菌免疫或非免疫猪的血清学变化。

Groups 组别	Assay 检测方法	Number of positive animals / tota lnumber of animals evaluated 阳性动物数/评估的总动物数					
		Experimental days 实验天数					
		D0 第0天	D7 第7天	D14 第14天	D21 第21天	D35 第35天	D55* 第55天*
G1 –Vaccinated 第1组 - 接种组	FCAT	0/8	4/8	6/8	8/8	8/8	4/4
	IPMA	1/8	4/8	4/8	8/8	8/8	4/4
G2– Non-vaccinated 第2组 - 未接种组	FCAT	0/8	0/8	0/8	0/8	0/8	0/4
	IPMA	0/8	2/8	4/8	1/8	0/8	0/4

*At D55, only 4 animals from each group were analyzed, since the others were sacrificed at previous moments for the analysis of other parameters (data not shown). The presence of anti-*L. intracellularis* IgG in pig's serum was evaluated by FCAT and IPMA.
*在第55天，每组仅分析了4只动物，因为其他动物此前已被安乐死以便分析其他参数（数据未显示）。通过FCAT和IPMA对猪血清中抗胞内劳森菌IgG的存在进行评估。

Course of the Disease
病程



Epidemiology流行病学

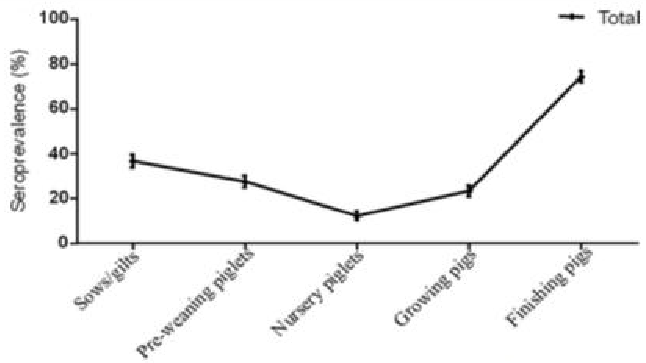


Fig. 1 Serological profile for *L. intracellularis* antibodies in swine herds from Minas Gerais state, Brazil. The bars indicate the standard error for the prevalence in each category

图1：巴西米纳斯吉拉斯州猪群中胞内劳森菌抗体的血清学特征。竖条表示每个类别中患病率的标准误差。

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

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*]

巴西米纳斯吉拉斯州猪群中胞内劳森菌感染的血清学特征、血清流行率和相关风险因素

- Risk factors 风险因素

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"与胞内劳森菌群体水平的血清阳性率显著相关。

Variable 变量	P value P值	Regression coefficient 回归系数	Standard error 标准误差	CI 置信区间 min 最小值	 max 最大值
New born assistance at farrow and piglets' first colostrum intake 在产仔时协助新生仔猪，并确保仔猪首次摄取初乳	0.22	0.17	0.14	−0.11	0.45
Crossfostering management 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 所使用的消毒剂数量	0.11	0.01	0.04	−0.07	0.09

CI-Confidence interval
CI-置信区间

Epidemiology流行病学

BMC Veterinary Research

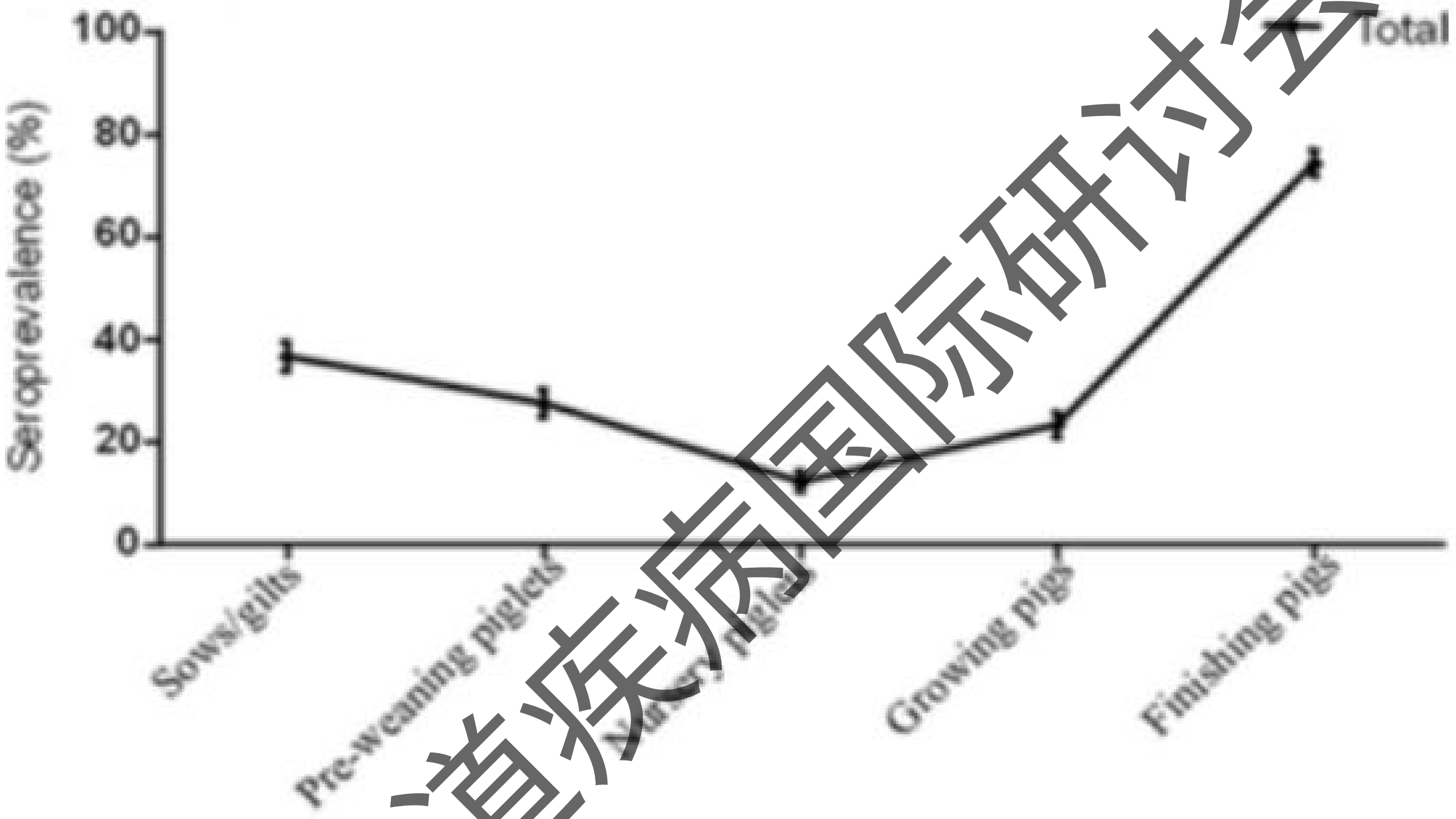


Fig. 1 Serological profile for *L. intracellularis* antibodies in swine herds from Minas Gerais state, Brazil. The bars indicate the standard error for the prevalence in each category

图1：巴西米纳斯吉拉斯州猪群中胞内劳森菌抗体的血清学特征。竖条表示每个类别中患病率的标准误差。

UFMG

Prevention and control
预防和控制

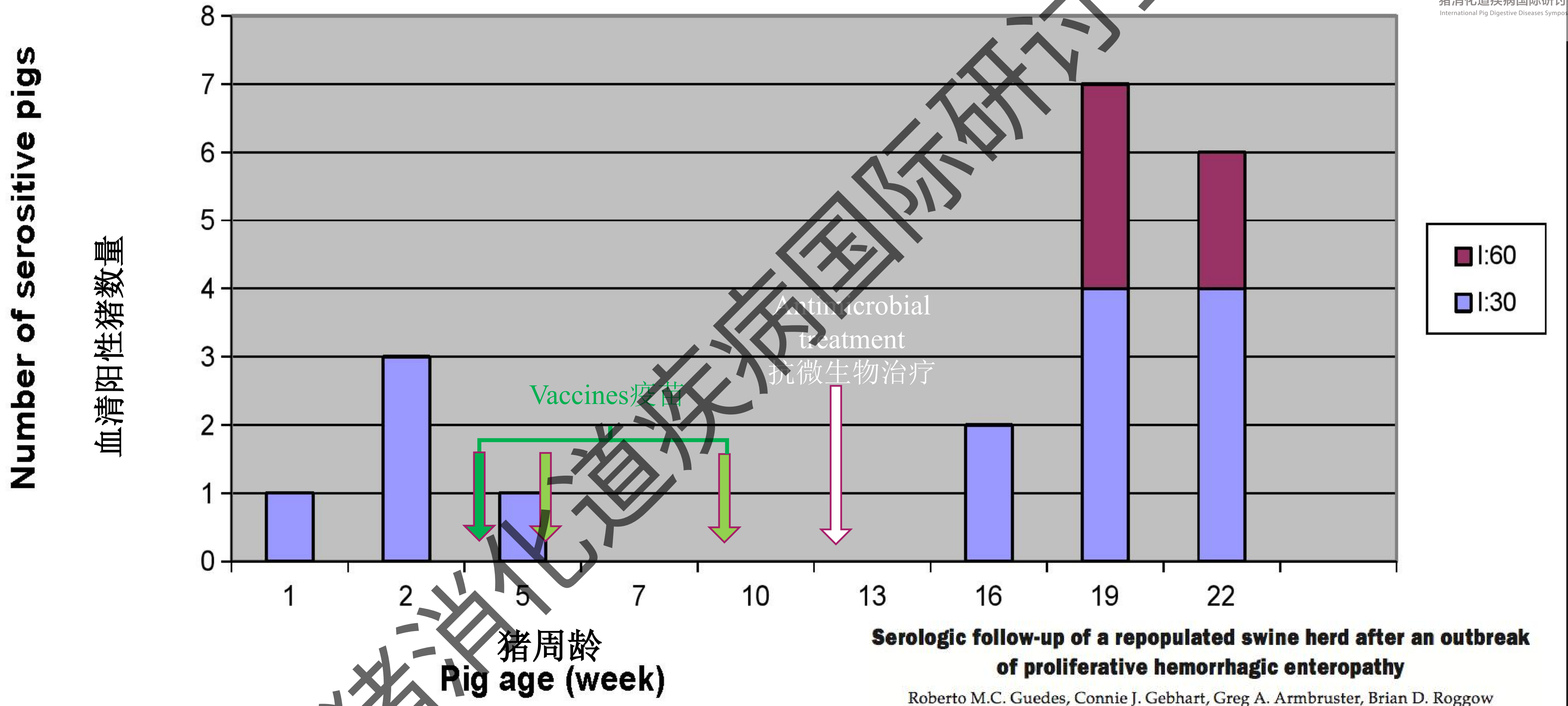
- **Treatment:**
- 治疗：
  - **Injectable (clinically sick animals), water soluble and in-feed medication**
    对于临床患病动物，采用注射剂、水溶性药物和饲料加药进行治疗
- **Prevention:**
  预防措施：
  - **Continuous use of in-feed medication (Restrictions and cost)**
    持续进行饲料加药（但存在一定限制和成本问题）
  - **"Immunological windows" "免疫窗口期"**
    - **Pulse medication (7 to 14 days), interval (3 to 4 weeks) (beginning of grow-finishing), new pulse medication**
      脉冲式用药（每隔7至14天进行一次），间隔3至4周（生长-育肥开始阶段），之后再进行新一轮脉冲式用药
    - **"Window" at the nursery 保育阶段的"窗口期"**
    - **Single pulse medication, based on herd seroprofile (or oral fluid)**
      基于猪群血清谱（或口腔液）进行单脉冲用药
  - Alternatives - Nutraceuticals 替代方案——营养保健品
  - Vaccine – Modified-live (Enterisol® Ileitis, Boehringer Ingelheim – Oral/Nasal)
    疫苗——改良活疫苗（Enterisol® Ileitis，勃林格殷格翰——口服/鼻腔）
  - Vaccine - Killed (Porcilis Ileitis, Merck Animal Health– IM)
    疫苗——灭活疫苗（Porcilis Ileitis，默克动物健康——肌肉注射）
  - Vaccine new technologies …. 新技术疫苗……

UFMG

Prevention and control
预防和控制

- **Treatment:**
  治疗：
  - **Injectable (clinically sick animals), water soluble and in-feed medication**
    对于临床患病动物，采用注射剂、水溶性药物和饲料加药进行治疗
- **Prevention:**
  预防措施：
  - **Continuous use of in-feed medication (Restrictions and cost)**
    持续进行饲料加药（但存在一定限制和成本问题）
  - **"Immunological windows" "免疫窗口期"**
    - **Pulse medication (7 to 14 days), interval (3 to 4 weeks) (beginning of grow-finishing), new pulse medication**
      脉冲式用药（每隔7至14天进行一次），间隔3至4周（生长-育肥开始阶段），之后再进行新一轮脉冲式用药
    - **"Window" at the nursery 保育阶段的"窗口期"**
    - **Single pulse medication, based on herd seroprofile (or oral fluid)**
      基于猪群血清谱（或口腔液）进行单脉冲用药
  - Alternatives - Nutraceuticals
    替代方案——营养保健品
  - Vaccine – Modified-live (Enterisol® Ileitis, Boehringer Ingelheim – Oral/Nasal)
    疫苗——改良活疫苗（Enterisol® Ileitis，勃林格殷格翰——口服/鼻腔）
  - Vaccine - Killed (Porcilis Ileitis, Merck Animal Health– IM)
    疫苗——灭活疫苗（Porcilis Ileitis，默克动物健康——肌肉注射）
  - Vaccine new technologies …. 新技术疫苗……

Seroprofile 血清谱



Serologic follow-up of a repopulated swine herd after an outbreak of proliferative hemorrhagic enteropathy

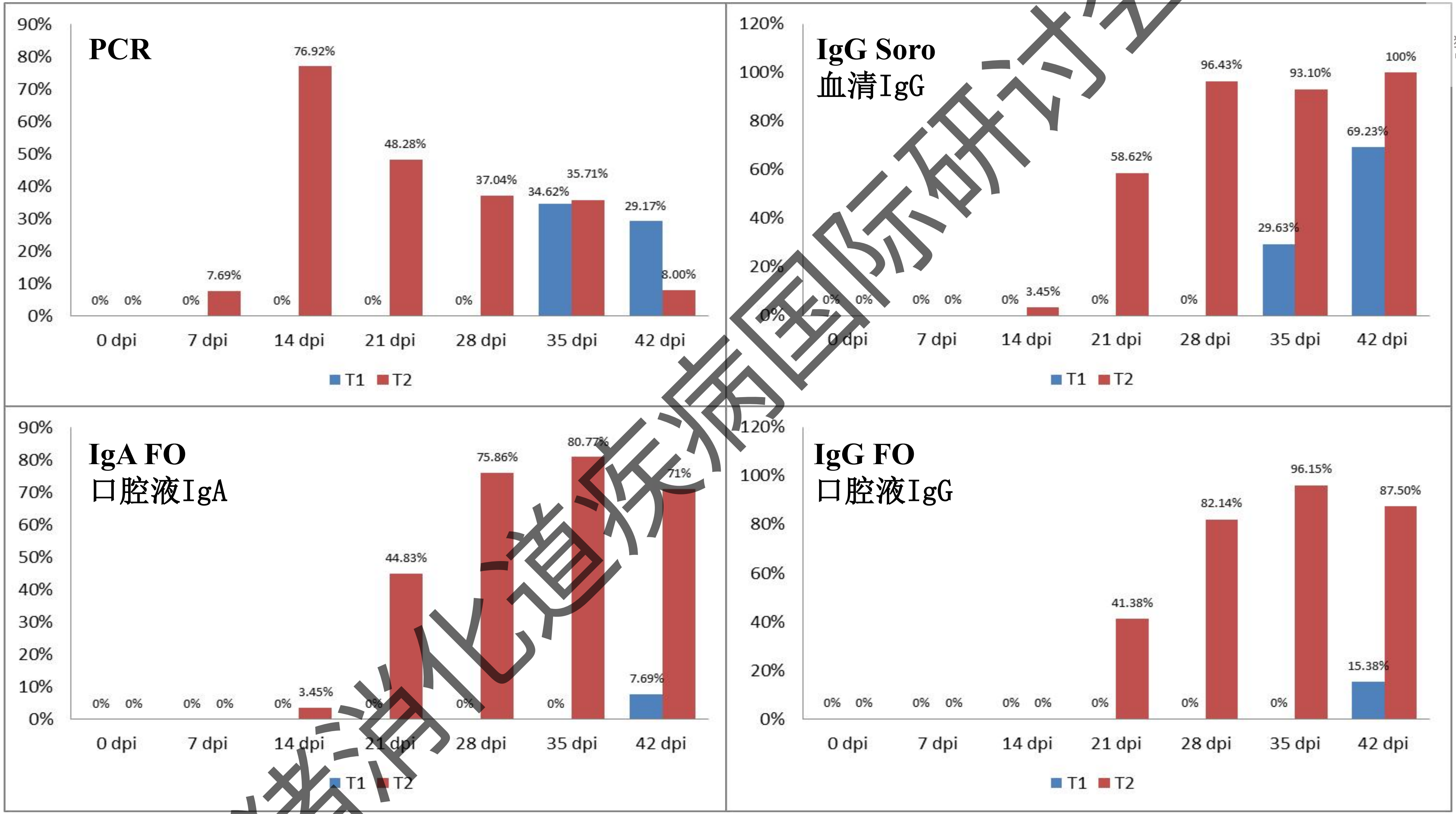
在猪群增生性出血性回肠炎暴发且重新补栏后的血清学追踪

Roberto M.C. Guedes, Connie J. Gebhart, Greg A. Armbruster, Brian D. Roggow

The Canadian Journal of Veterinary Research

2002;66:258-263

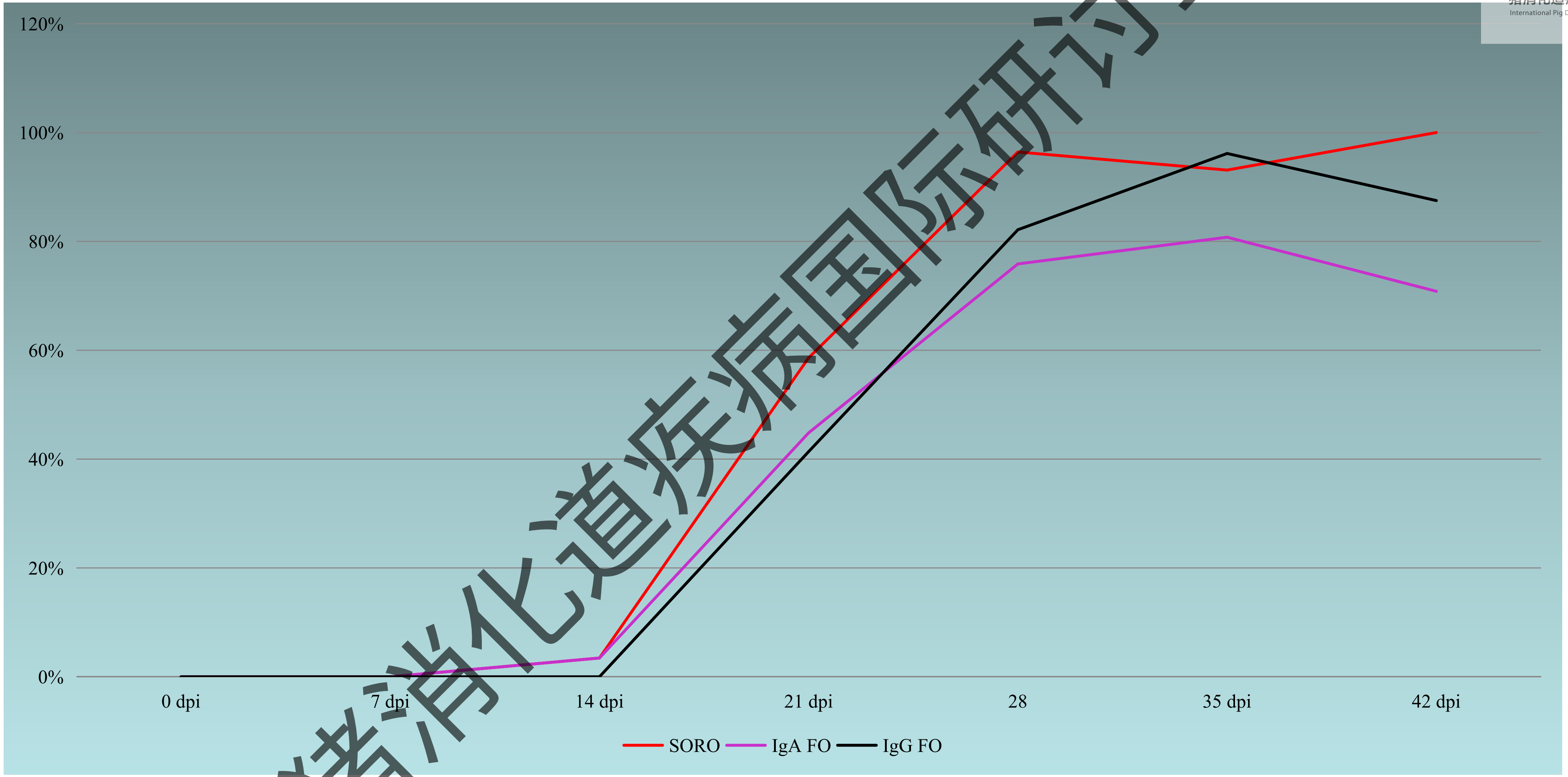
Detection of IgA and IgG anti-*Lawsonia intracellularis* in oral fluids of experimentally infected pigs
口腔液中胞内劳森菌特异性IgA和IgG的检测



Gabardo, 2014; Gabardo et al, Pat. Vet. Bras., em revisão
Gabardo, 2014; Gabardo等，Pat. Vet. Bras.，审阅中

UFMG

Percentage of serum IgG, and IgG and IgA in oral fluids in positive pigs
阳性猪血清中的IgG和口腔液中的IgG和IgA百分比

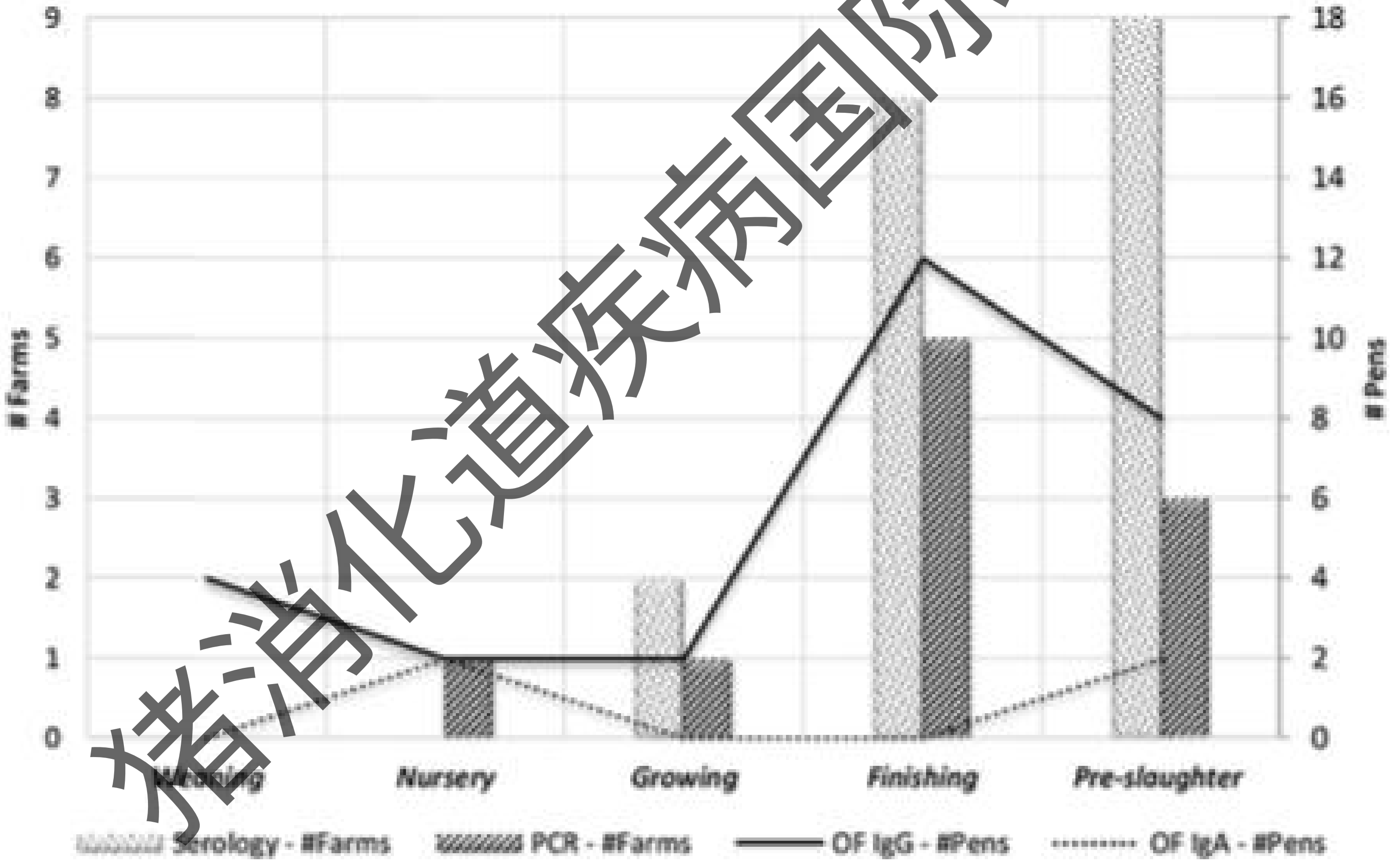


Gabardo, 2014; Gabardo et al, Pat. Vet. Bras., em revisão
Gabardo, 2014; Gabardo等，Pat. Vet. Bras.，审阅中

Oral fluid for detection of exposure to *Lawsonia intracellularis* in naturally infected pigs 用于检测自然感染猪细胞内劳森氏菌接触水平的口腔液

Javier A. Barrera-Zarate, Mariana R. Andrade, Carlos E.R. Pereira, Amanda Vasconcellos, Mariane M. Wagatsuma, Jose P.H. Sato, Amanda Gabrielle S. Daniel, Lucas A. Rezende, Luisa A.V. Otoni, Ricardo P. Laub, Nubia R. Macedo, Camila M. Costa, João Paulo A. Haddad, Roberto M.C. Guedes*

The Veterinary Journal 244 (2019) 34–36



UFMG

Final considerations
最终结论

- There are many different serological tests available

  可采用多种不同的血清学检测方法

- Serology is an interesting tool for understanding the kinectic of *L. intracellularis* infection

  血清学是了解胞内劳森菌感染动态的有趣工具

- Farrow-to-finish on-site farms are easier to interpret the results compared to multiple sites with different origins

  与来自不同地区的多点式养殖场相比，一点式产仔至育肥养殖场内更容易解释研究结果

Swine pathology group 猪病理学研究组

Acknowledgements
致谢

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

CAPES
CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico
UFMG
Escola de Veterinária UFMG
FAPEMIG