# 泰万菌素：猪蓝耳病防控的新选择



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# 提纲

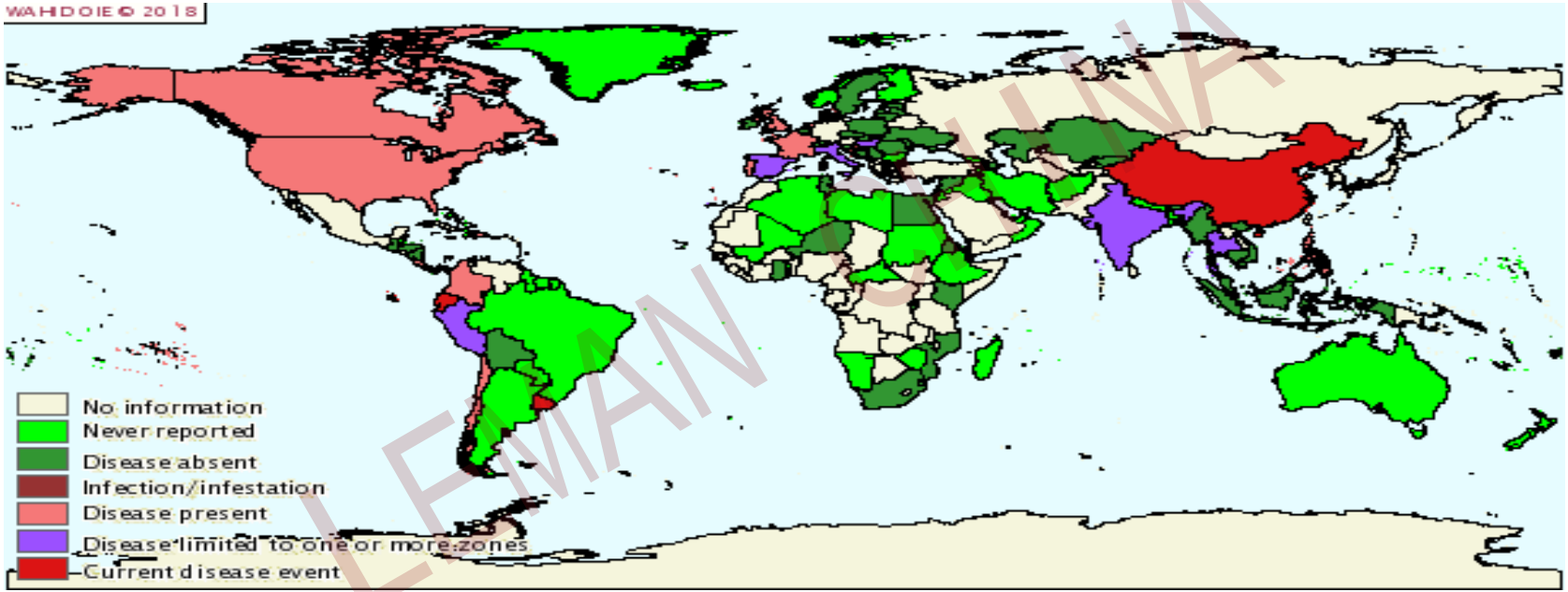
- 蓝耳病：全球流行的猪病
- 蓝耳病防控的挑战
- 抗蓝耳病病毒感染的探索

# 1.

## 蓝耳病：全球流行的猪病

- 蓝耳病1987年发现于美国，1991在荷兰鉴定出病毒，然后传播到世界各地。
- 该病1995年在中国北方首次暴发，2-3年传播到全国。
- 2006年中国首先出现高致病性毒株，2014年相继在美国和中国出现另一种高致病性毒株NADC30 和NADC30-like。
- 临床上，感染猪的移动是该病最常见的传播方式。

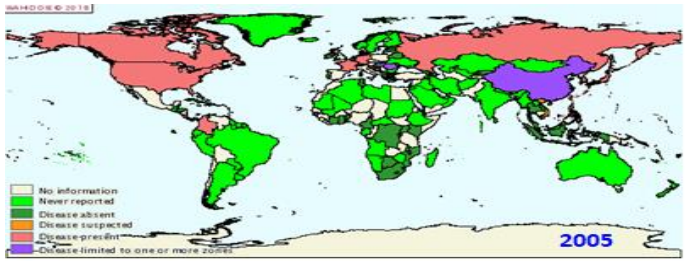
# 2017年猪蓝耳病的世界分布图

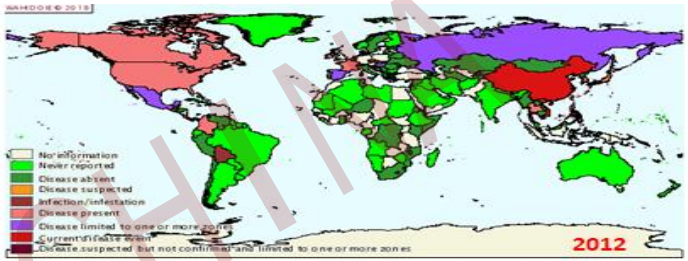


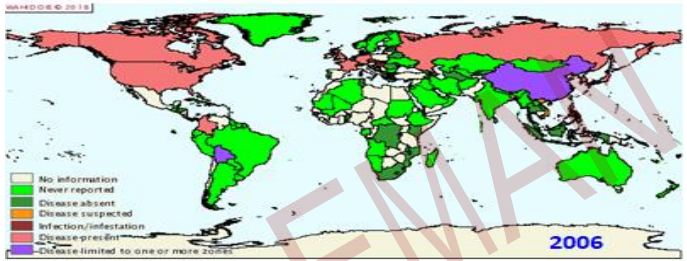


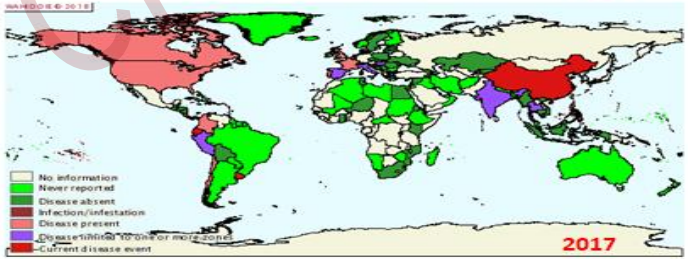

www.oie.int

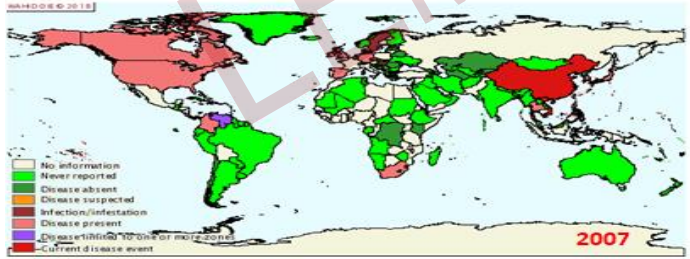
# 2007年以来中国成为蓝耳病最活跃的地区

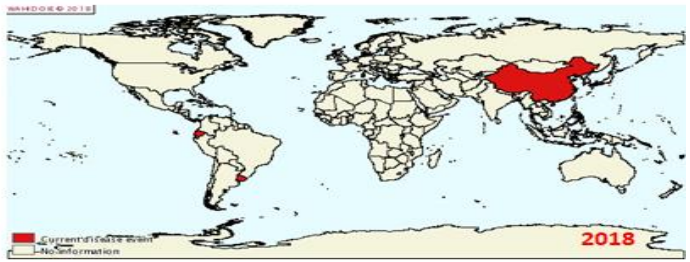


# Estimated economic lost 经济损失估算

- **USA 美国**
  - USD 560-663 million/year, USD 121/sow
    5.6-6.6亿美元/年，121美元/头母猪
- **Europe 欧洲**
  - EURO 126/sow 126欧元/头母猪
- **China 中国**
  - About 35-37 million sows 大约3500-3700万头母猪
  - USD 3.5-3.7 billion/year (USA 100/sow)
    35-37亿美元/年（100美元/头母猪）



[1] Neumann et al., J Am Vet Med Assoc 2005
[2] Holtkamp et al., J Swine Health Prod 2013
[3] Nieuwenhuis et al., Vet Rec 2012

# 2. Challenge on PRRS control
## 蓝耳病防控的挑战

### Current control strategies on PRRS 当前的蓝耳病防控策略

- Vaccination 疫苗接种
- Control secondary bacterial infection 控制细菌继发感染
- Exposure to PRRSV: serum inoculation, contact with infected pigs 驯化
- Herd depopulation and repopulation 清除阳性猪群、重建阴性猪群
- Test, removal and herd closure 检测、淘汰和封群
- Anti-PRRSV drugs? 抗病毒药物?

**So far, no single 'sovereign remedy' has been found for PRRS.**

**迄今没有对蓝耳病有效的“灵丹妙药”！**

Zhou & Yang. Virus Res 2010

# Challenge on PRRS control

## 蓝耳病防控的挑战

- High diversity of PRRSV caused by frequent mutation and recombination 病毒的广泛变异与重组，新毒株层出不穷
- Immunosuppressive and secondary infections 免疫抑制与继发感染
- Uncertainty of the efficacy and safety of PRRS vaccines 疫苗安全性和有效性的不确定
- Lack of anti-PRRSV drugs 缺乏有效防治药物
- Slow progress on PRRS eradication programs in farms and regions 猪场与区域净化进展缓慢

# 3. Anti-viral approaches against PRRSv

## 抗蓝耳病病毒感染的探索

- Virus entry blockers 病毒侵入阻断剂
- MicroRNAs and antisense RNA based strategy 小RNA和反义RNA
- PRRSv neutralizing targets 病毒中和靶位
- Immune stimulators 免疫激活剂
- Herbal extracts and chemical compounds 植物提取物和化合物
- Antibiotic macrolides (tilmicosin and tylvalosin) 大环内酯类抗生素(替米考星和泰万菌素)

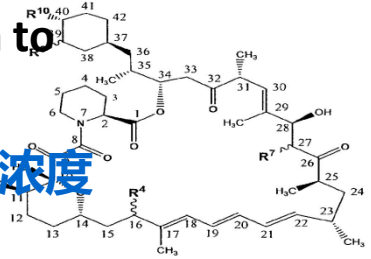


Du et al., Trends Microbiol 2017

# Antibiotic macrolides

## 大环内酯类抗生素

- belong to protein synthesis inhibitors 蛋白质合成抑制剂
- used to treat infections caused by Gram-positive bacteria and limited Gram-negatives, and some respiratory tract and soft-tissue infections. 主要用于革兰氏阳性菌和部分革兰氏阴性菌感染的治疗，适用于呼吸道和软组织的感染。
- have broad immune-modulating & anti-inflammation properties 广泛的免疫调节和抗炎活性
- accumulate with high affinity in phagocytes and has been shown to reach intracellular concentrations up to 500 times greater than systemic levels. 对巨噬细胞具有高亲和力而在包内聚集，胞内浓度可以达到血药浓度的500倍。



# Tylvalosin: anti-PRRSv effect?

## 泰万菌素抗蓝耳病?

- the third-generation macrolides. 第三代大环内酯类抗生素
- broader antibacterial spectrum, lower toxicity. 更广谱，毒性低
- PRRSv is an immune dysregulatory disease, and mainly replicates in macrophages. 蓝耳病是免疫抑制性疾病，病毒主要在巨噬细胞中复制
- Tylvalosin: anti-PRRSv effect? 抗蓝耳病病毒作用?

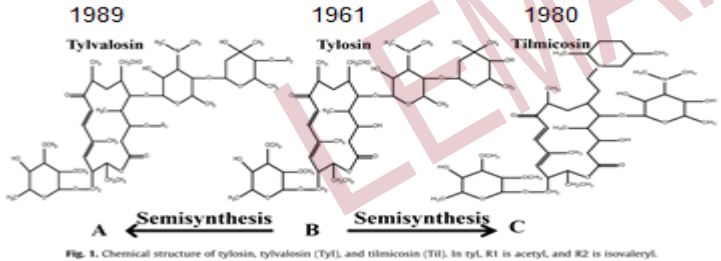
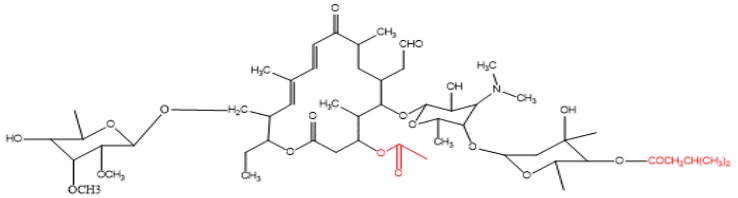



Fig. 1. Chemical structure of tylosin, tylvalosin (Tyl), and tilmicosin (Til). In tyl, R1 is acetyl, and R2 is isovaleryl.

tylosin derivatives 泰乐菌素衍生物



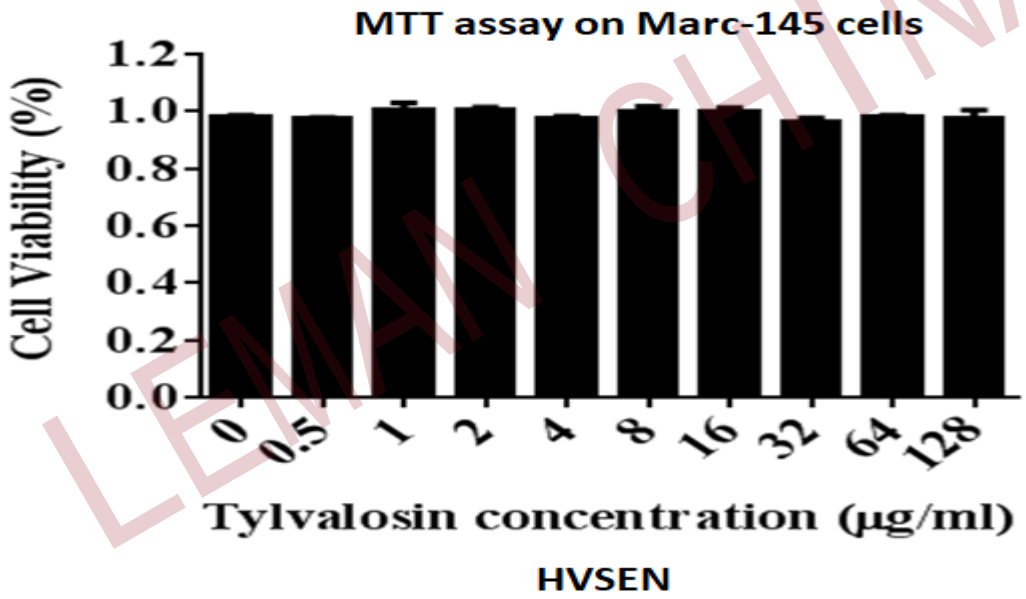
tylvalosin (acetylisovaleryl tylosin tartrate)
泰万菌素(酒石酸乙酰异戊酰泰乐菌素)

# 3.1 *in vitro* experiments
## 体外实验

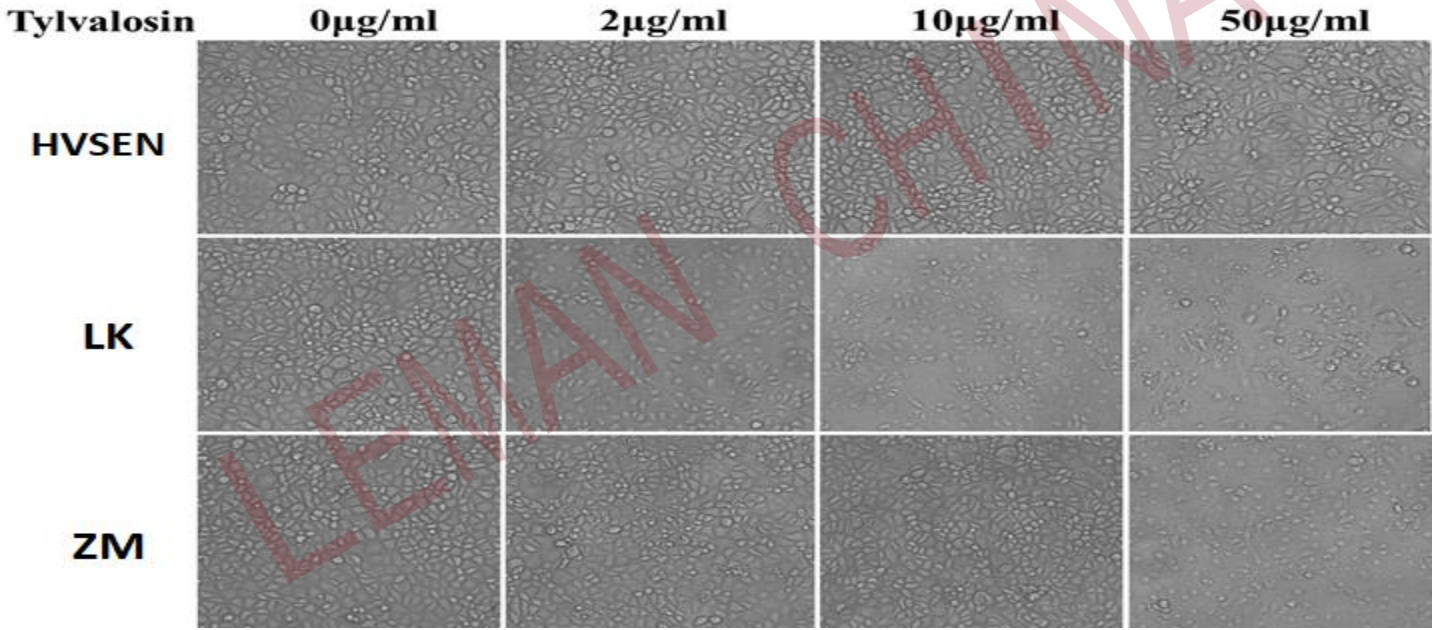
- Cytotoxicity of tylvalosin 泰万菌素的细胞毒性
- Inhibition of PRRSV replication 泰万菌素抑制PRRSV复制
- Inhibition of PRRSV-induced inflammation 泰万菌素抑制PRRSV诱导的炎症反应

# Cytotoxicity of tylvalosin

## 泰万菌素的细胞毒性

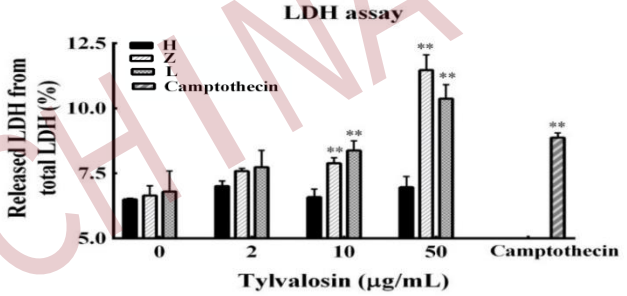


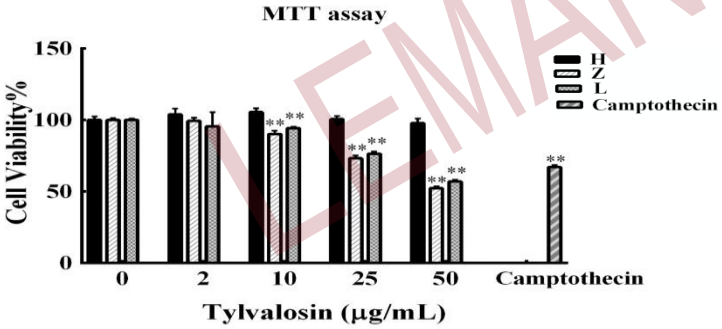
# Cytotoxicity of tylvalosin from different sources
# 不同来源的泰万菌素的细胞毒性不同

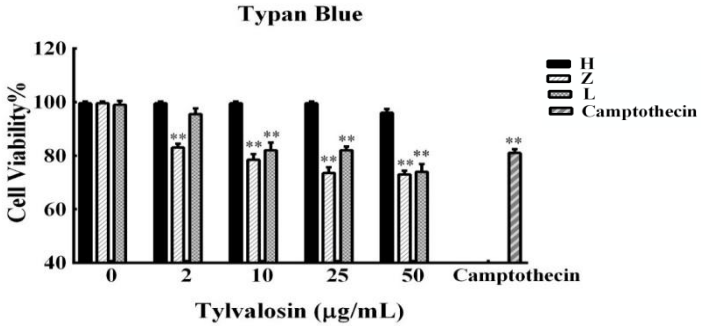


# Cytotoxicity of tylvalosin from different sources
# 不同来源的泰万菌素的细胞毒性不同

Cell proliferation
细胞增殖活性

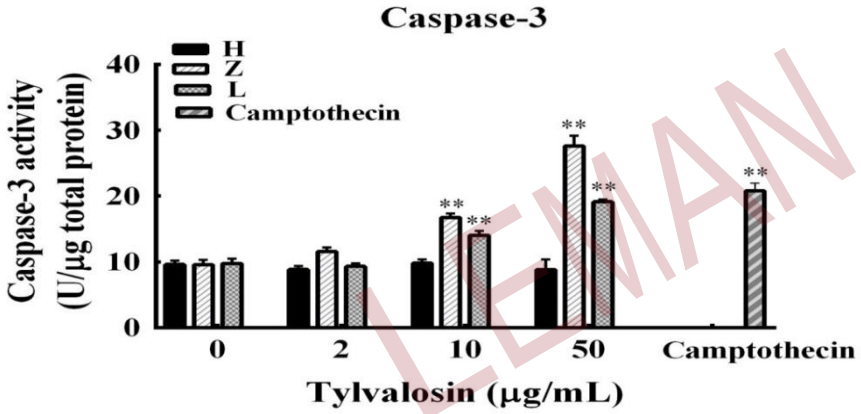


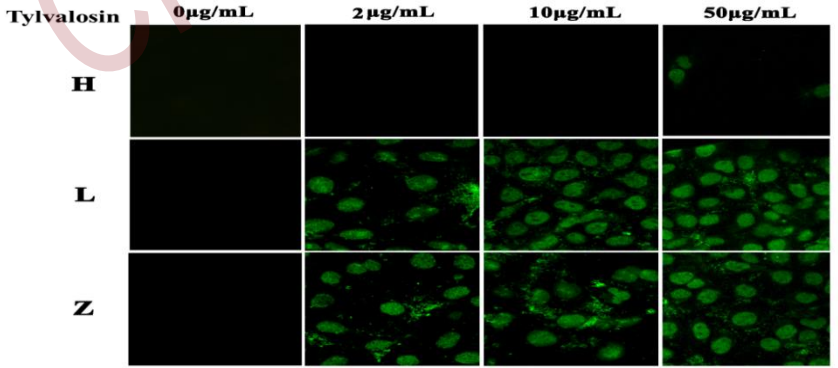



# Cytotoxicity of tylvalosin from different sources
# 不同来源的泰万菌素的细胞毒性不同

## Cell apoptosis
## 细胞凋亡



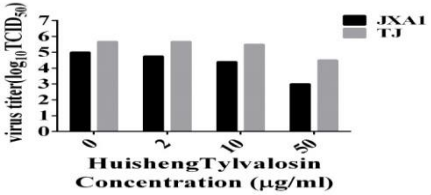
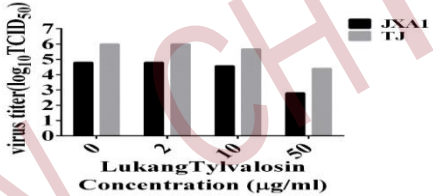
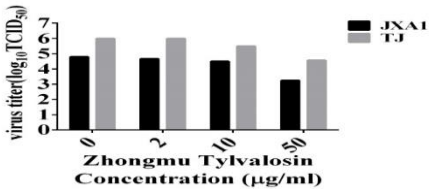
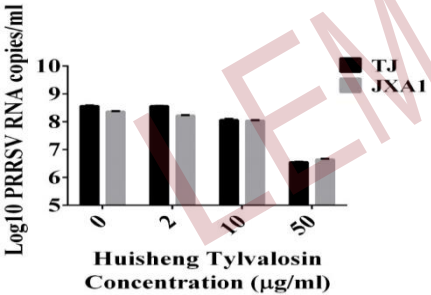
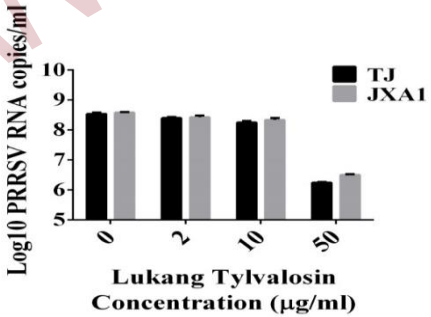
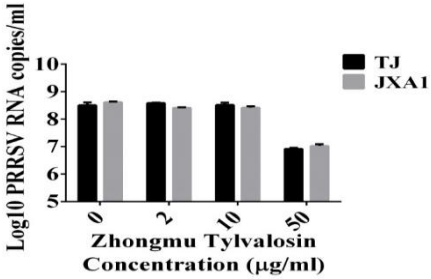
# Why different?
## 为什么细胞毒性不同？

| Retention time/min | HVSEN | LK | ZM |
|---|---|---|---|
| 2.414 | 0.98% | 1.00% | 1.78% |
| 2.649 | 0.11% | 0.0% | 0 |
| 2.919 | 1.08% | 0.63% | 0 |
| 3.671 | 0.44% | 1.29% | 0.24% |
| 5.193 | 1.29% | 1.19% | 0.92% |
| 5.433 | 1.68% | 1.72% | 1.32% |
| 5.849 | 0.04% | 0.68% | 0.55% |
| 6.235 | 3.99% | 3.70% | 3.65% |
| 6.973 | 2.48% | 1.35% | 3.52% |
| **7.868** | **85.41%** | **85.07%** | **86.08%** |
| 10.631 | 0.66% | 1.25% | 0.85% |
| 15.635 | 0.29% | 0.28% | 0.09% |
| 21.100 | 1.40% | 1.23% | 0.66% |
| 35.029 | 0.13% | 0.20% | 0.16% |

# Inhibition of PRRSV replication

## 泰万菌素抑制PRRSV复制



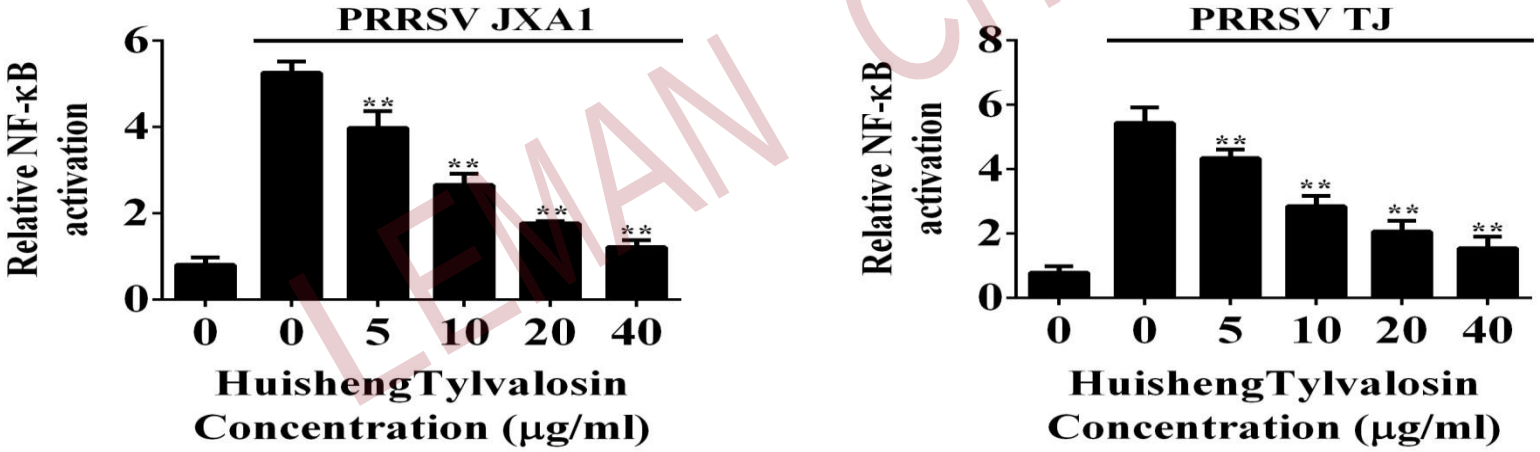
| | HVSEN | LK | ZM |
|---|---|---|---|
| TCID50 |  |  |  |
| qRT-PCR |  |  |  |

# Inhibition of PRRSV-induced inflammation

## 泰万菌素抑制PRRSV诱导的炎症反应

Inhibition PRRSV-induced NF-κB activity

抑制PRRSV诱导的NF-κB活性

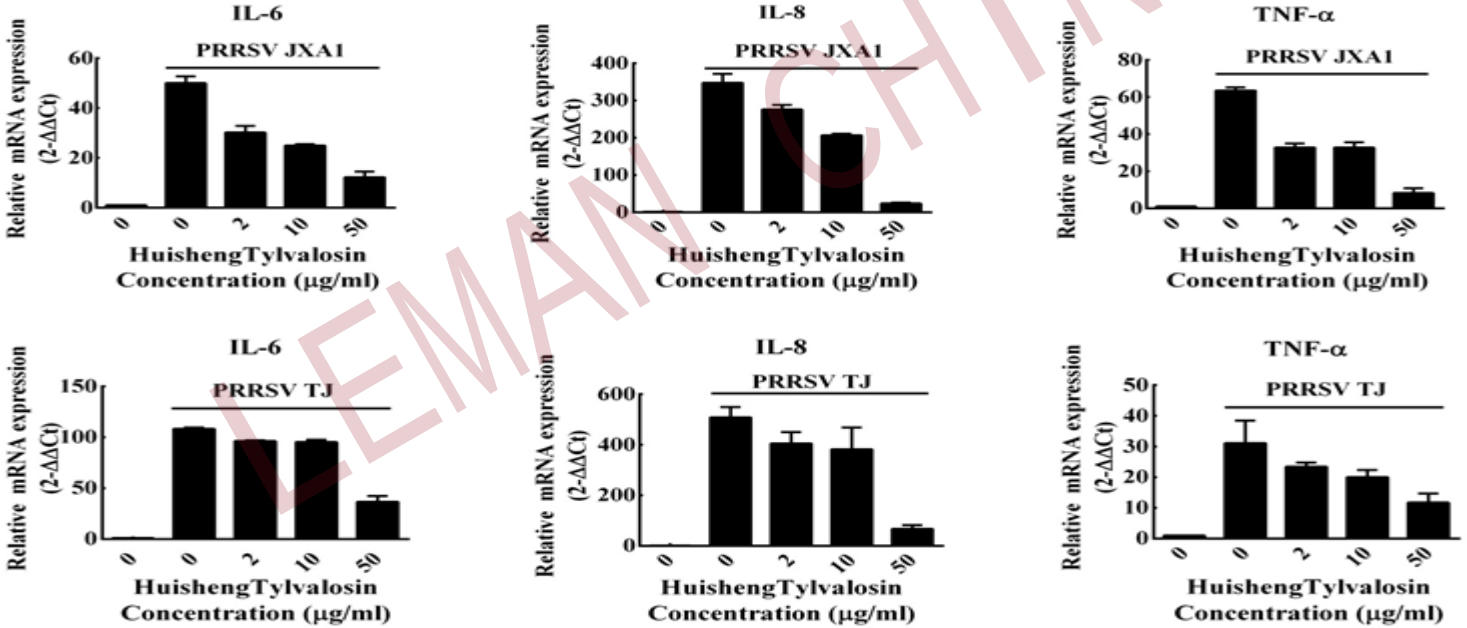


# Inhibition of PRRSV-induced inflammation

## 泰万菌素抑制PRRSV诱导的炎症反应

### Inhibition PRRSV-induced cytokines

抑制PRRSV诱导的炎性因子表达



# 3.2 animal experiments

## 猪体内试验

Groups

- JXA1-like （n=5）
- JXA1-like+Tylvalosin （n=5）
- NADC30-like （n=5）
- NADC30-like +Tylvalosin （n=5）
- Mock （n=5）

1-7 days

- Tylvalosin (5mg/kg BW)
- Rectal temperature
- Weight—D1,D7

Challenge

- Im. 3ml
- Intranasal 3ml ($10^5TCID_{50}$/ml)

9-17 days

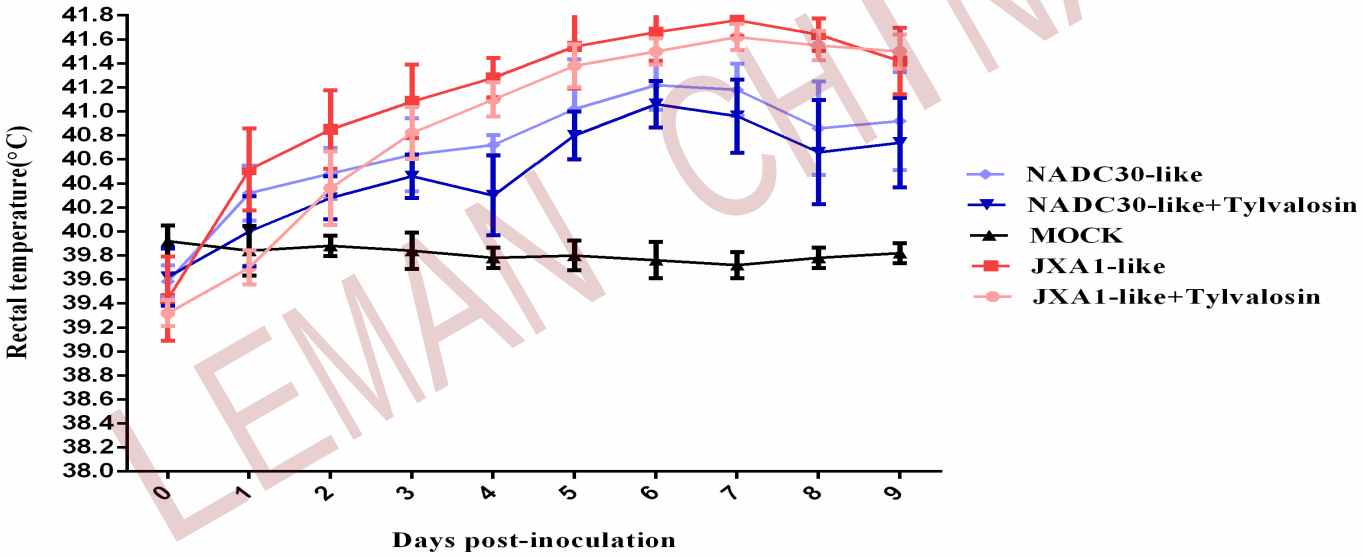
- Tylvalosin (5mg/kg BW)
- Rectal temperature
- Blood samples(anterior chamber)—DPI0,3,6,9
- Weight—D12,D17

Necropsy (DPI 9)

- Heart
- Liver
- Spleen
- Lung
- Kidney
- Tonsil
- Submaxillary
- Inguinal
- Brain
- Blood

# Reduce body temperature

## 抑制体温升高



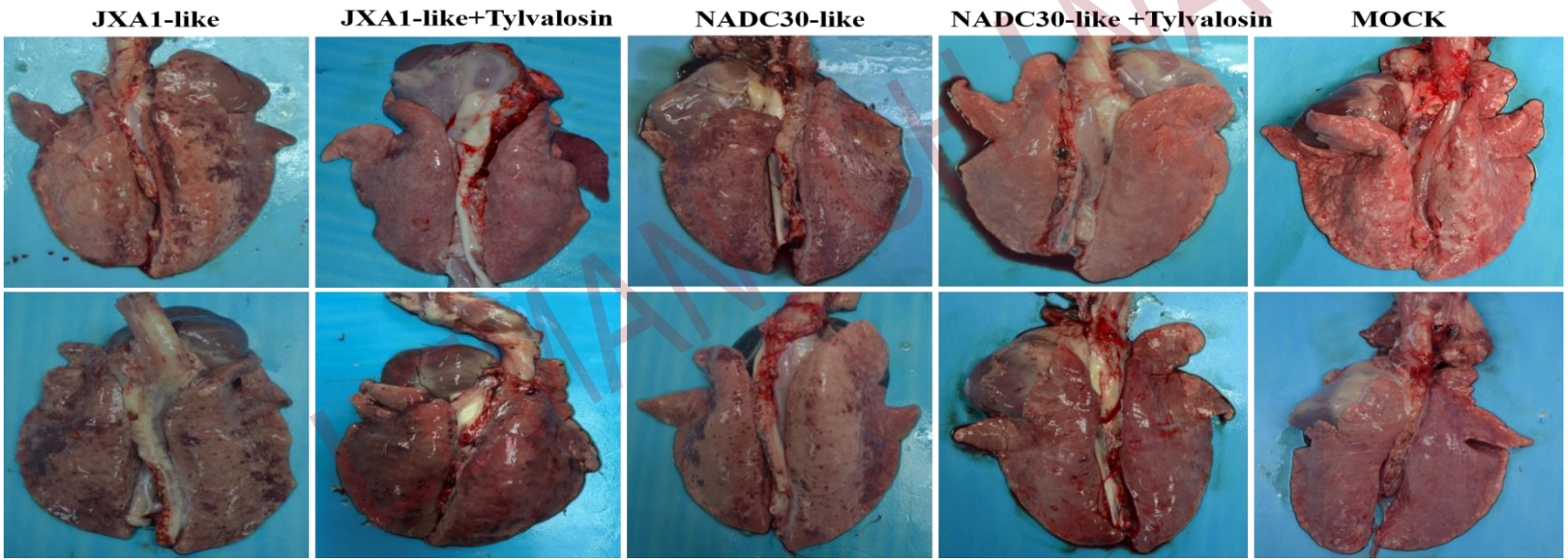
# Decrease body weight loss

## 降低猪日增重损失

| Group | Body weight (kg) | | | | Average body weight gain before challenge | Average body weight gain whole trial period |
|---|---|---|---|---|---|---|
| | D0 | D7 | D12 | D17 | | |
| JXA1-like | 5.15 | 6.20 | 6.66 | 6.65 | 0.15 | 0.37 |
| JXA1-like+Tylvalosin | 5.59 | 7.40 | 7.74 | 9.21 | 0.25 | 0.64 |
| NADC30-like | 4.94 | 6.03 | 7.31 | 7.01 | 0.16 | 0.42 |
| NADC30-like+Tylvalosin | 5.75 | 7.38 | 8.96 | 8.60 | 0.23 | 0.55 |
| Mock | 5.68 | 6.97 | 9.08 | 9.81 | 0.18 | 0.85 |

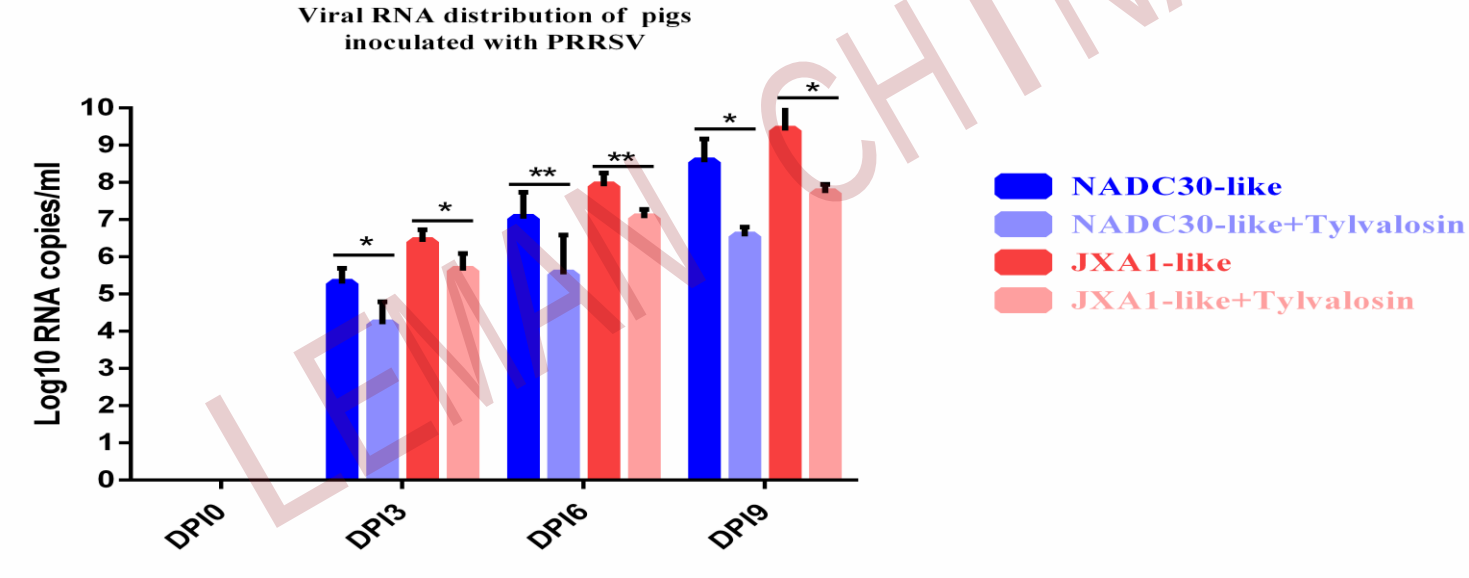
# Decrease lung lesion

## 减轻肺损伤



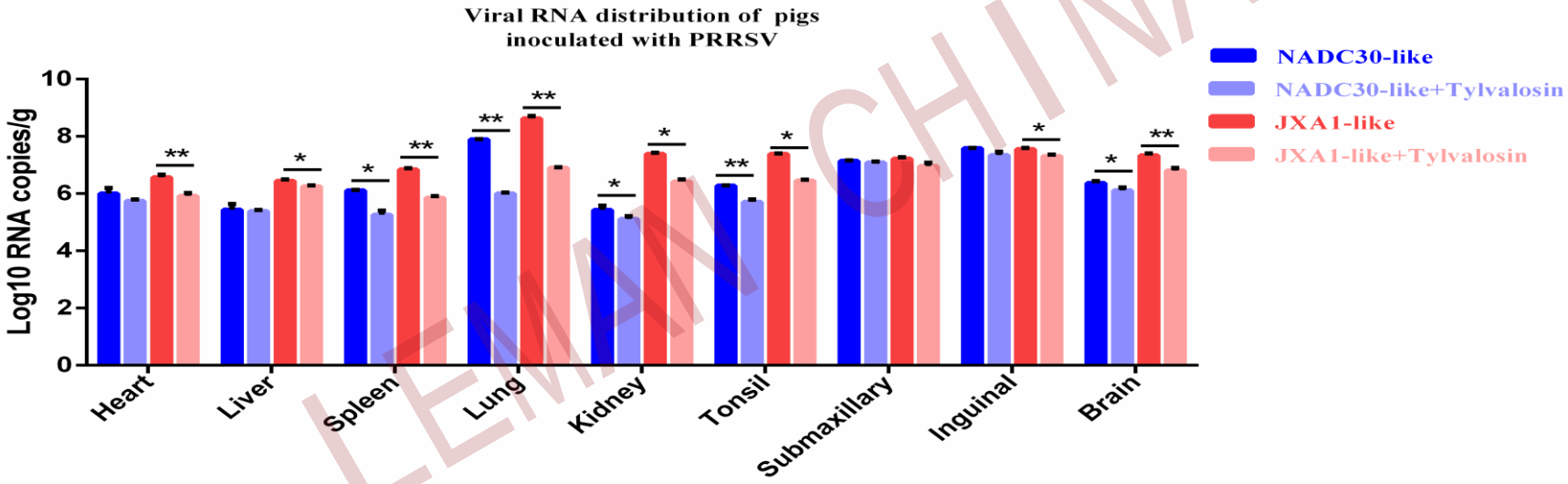
# Reduce PRRS virus load in the blood

## 降低PRRS病毒血症



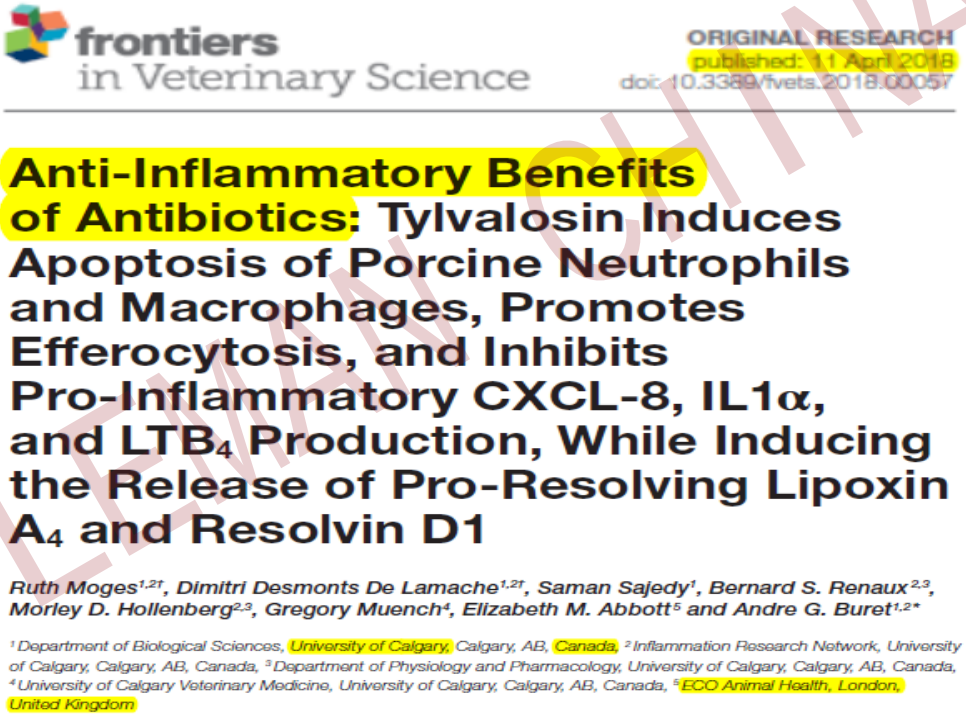
# Reduce PRRS virus load in different tissues

## 降低组织中的病毒载量



# Recent progress on the anti-inflammatory mechanism of tylvalosin

## 泰万菌素抗炎机制的最近研究进展



frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 11 April 2018
doi: 10.3389/fvets.2018.00057

**Anti-Inflammatory Benefits of Antibiotics: Tylvalosin Induces Apoptosis of Porcine Neutrophils and Macrophages, Promotes Efferocytosis, and Inhibits Pro-Inflammatory CXCL-8, IL1α, and $LTB_4$ Production, While Inducing the Release of Pro-Resolving Lipoxin $A_4$ and Resolvin D1**

*Ruth Moges[1,2†], Dimitri Desmonts De Lamache[1,2†], Saman Sajedy[1], Bernard S. Renaux[2,3], Morley D. Hollenberg[2,3], Gregory Muench[4], Elizabeth M. Abbott[5] and Andre G. Buret[1,2*]*

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# Take home message

## 小　结

- Tylvalosin can inhibit PRRSV replication in vitro and reduce virus load in infected pigs. 泰万菌素可以在体内外抑制蓝耳病病毒的复制，降低感染猪中的病毒载量。
- Tylvalosin can relieve PRRSV-induced inflammation and lung lesion. 泰万菌素可有效缓解蓝耳病病毒感染导致的炎症反应和肺损伤。
- Tylvalosin is a promising player on the control of PRRS, a globally spread pig pandemic. 泰万菌素是当前猪蓝耳病防控的新选择。

# Acknowledgement
# 致　谢



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Jie Liu

Xiugao Zhu

Wanyong Tang


# THANKS!