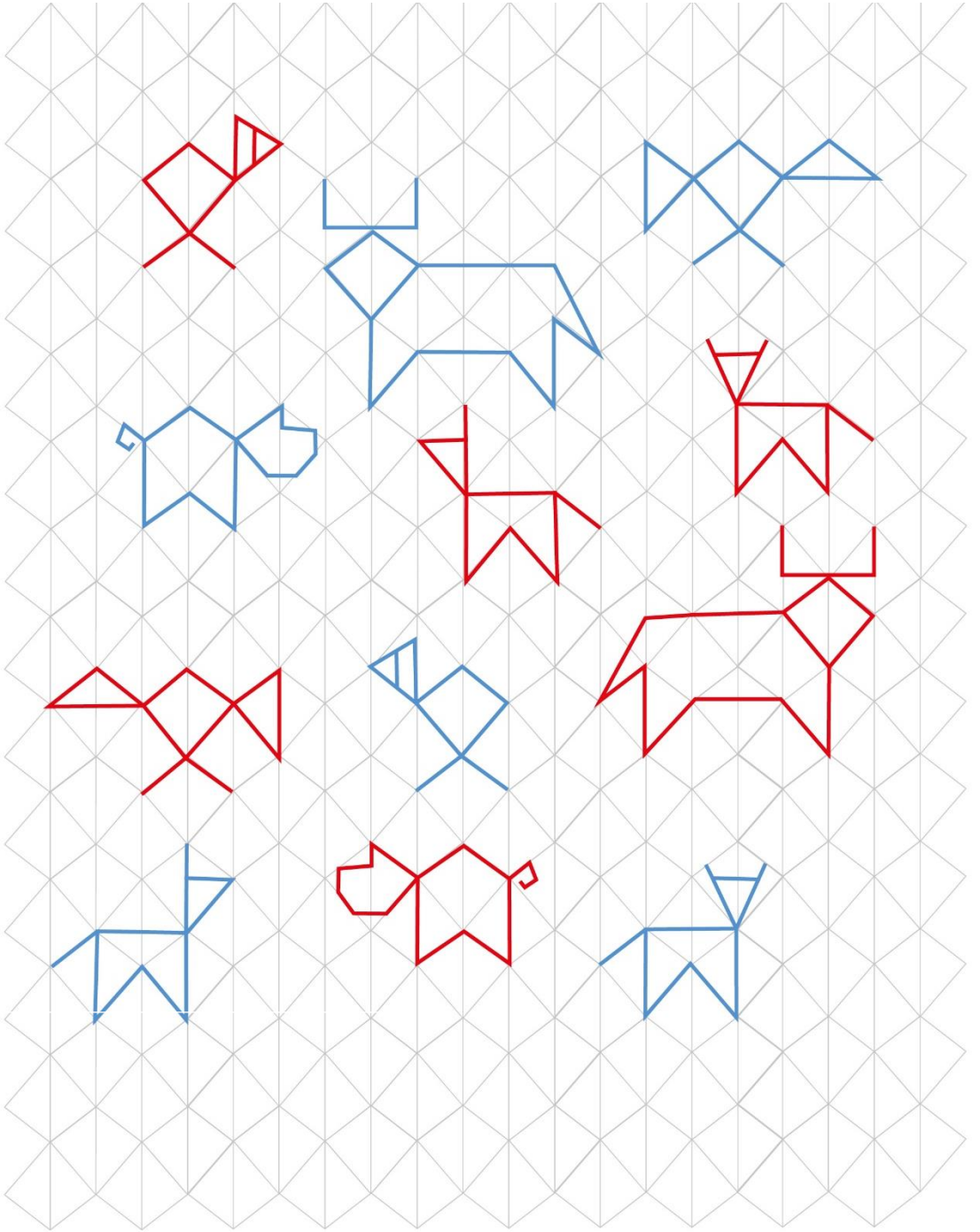
China, Mongolia, and Central Asia Episystem Workshop for Peste des petits ruminants (PPR) eradication

Ulaanbaatar, Mongolia, 1-3 April 2025



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Ministry of Agriculture and Rural Affairs of the People's Republic of China

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PPR Diagnostics:

The Role of PPR Reference Laboratory in Support of Eradication through Episystem Approach

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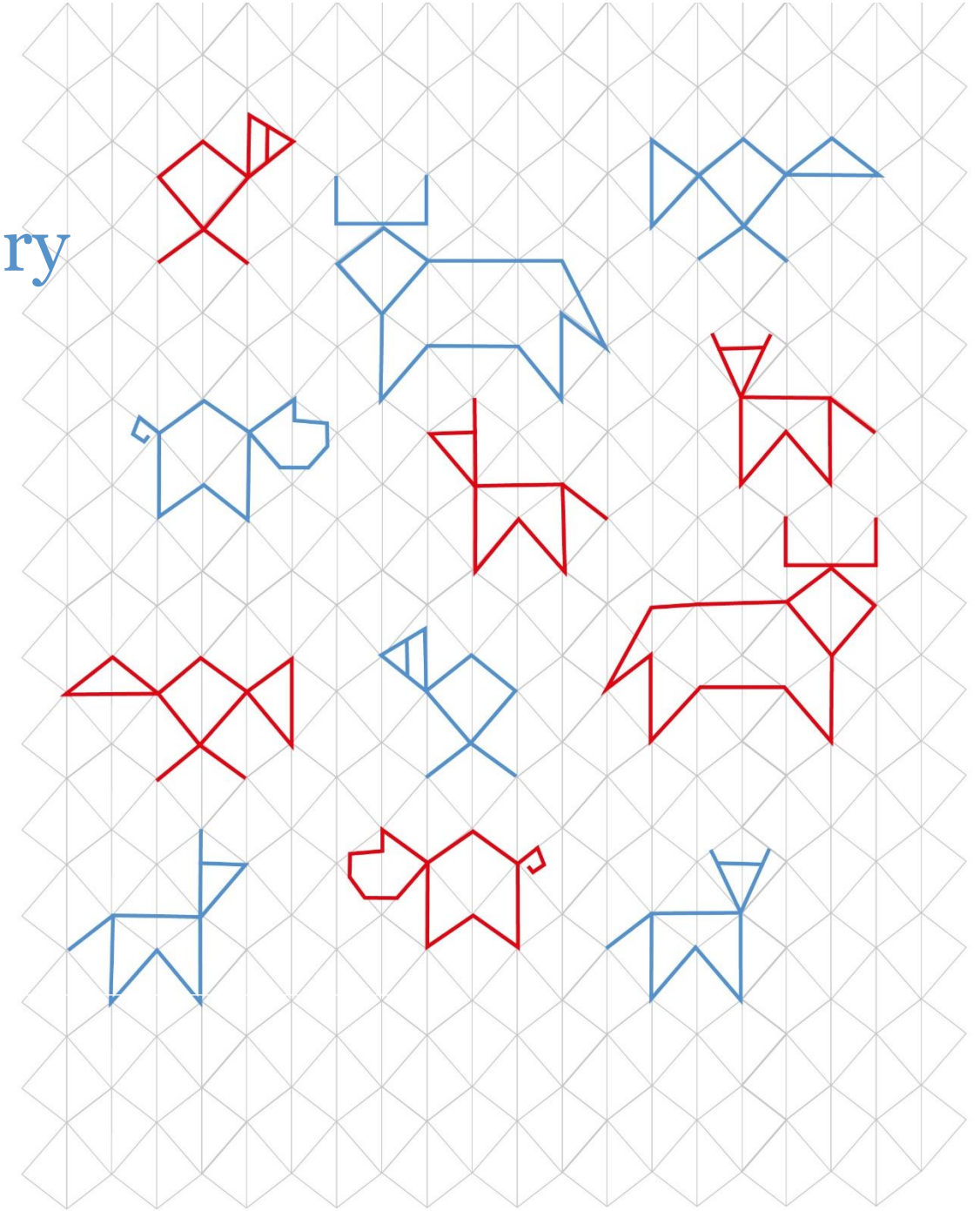
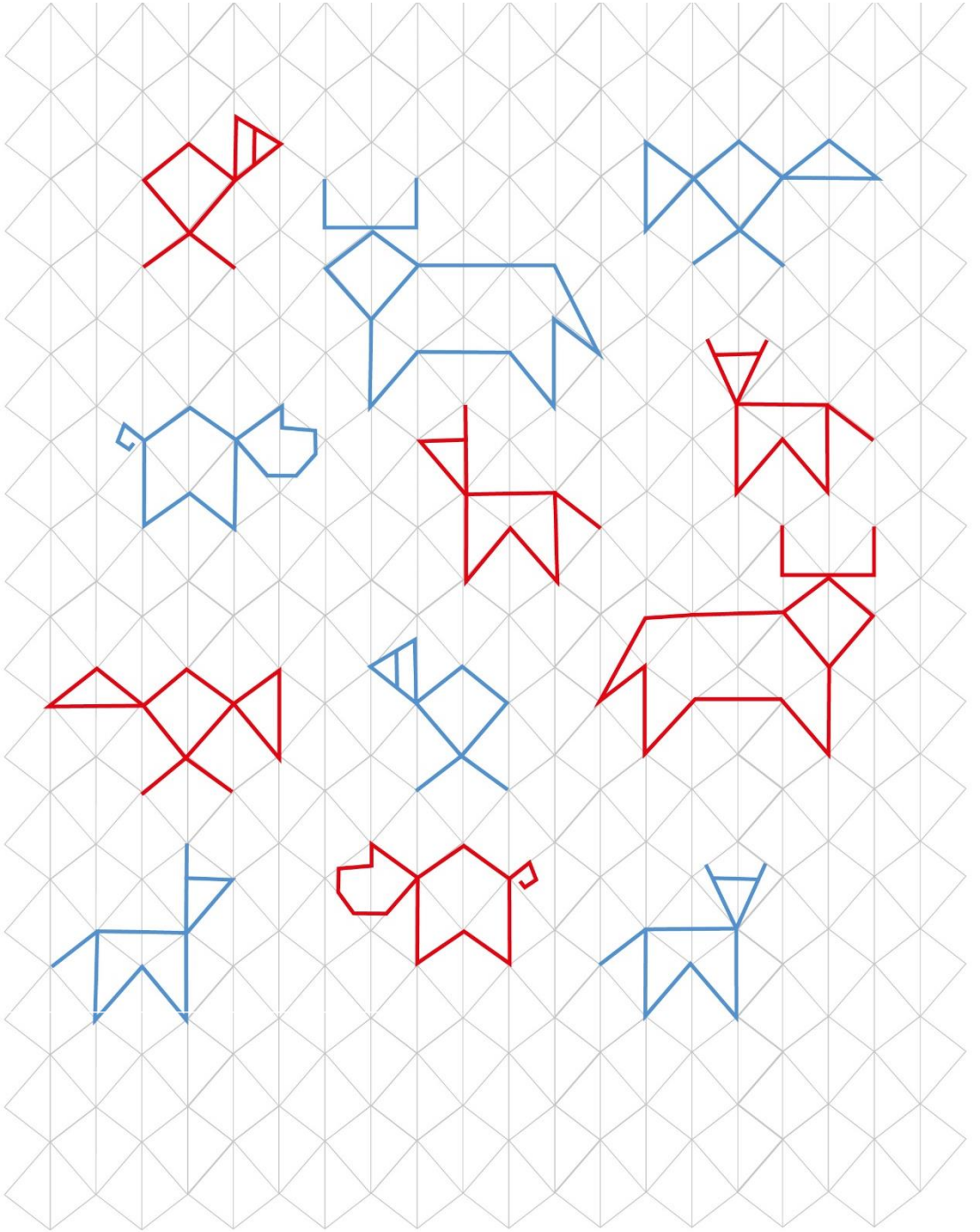


OUTLINES

- PPR Susceptible Animals
- PPR Diagnostics
- The Role of RL in PPR Episystem Approach



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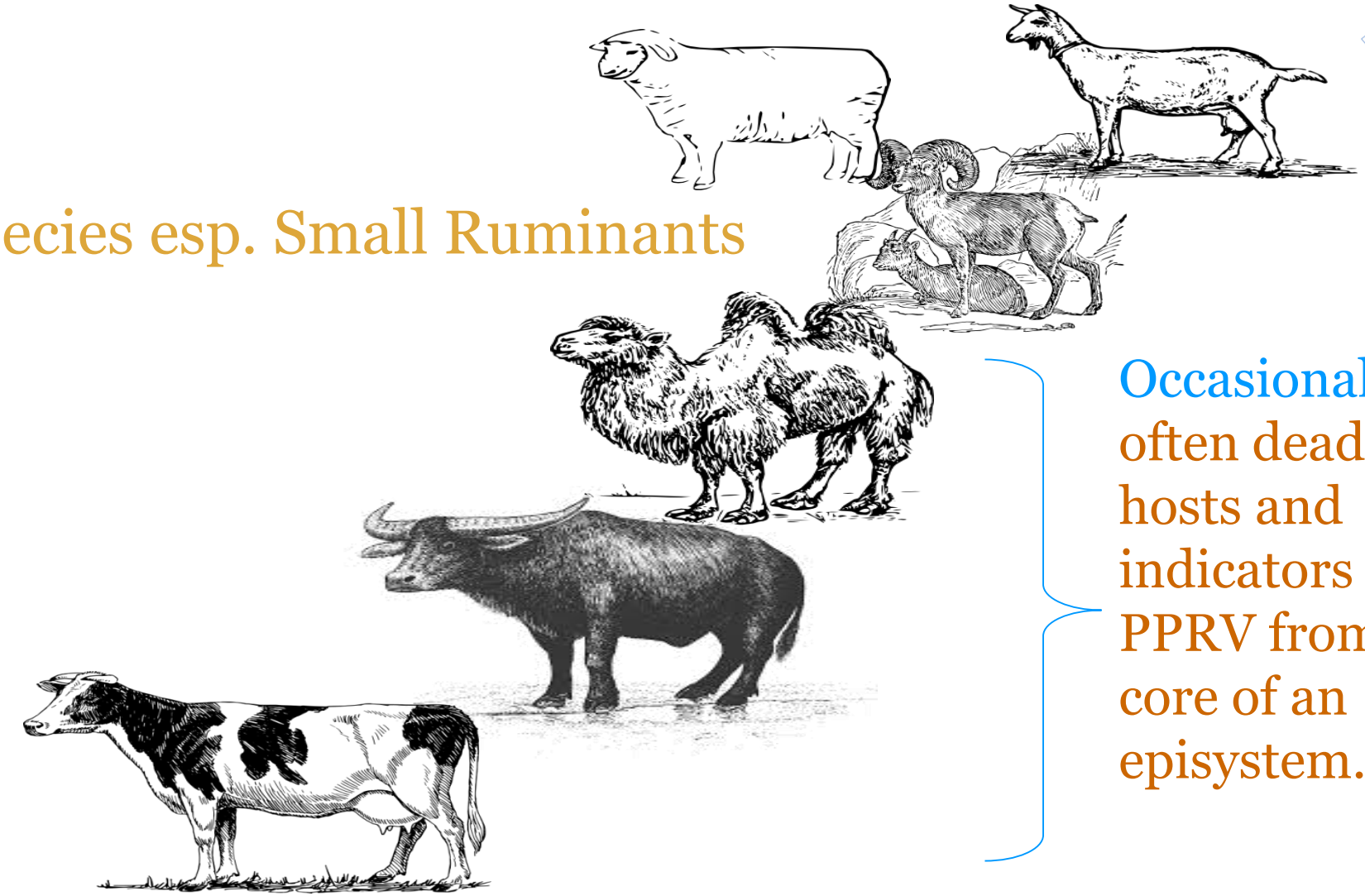
Section 1: PPR Susceptible Animals

Summary of PPR Susceptible Animals

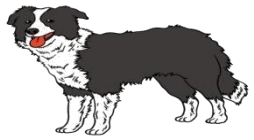
- ✓Sheep, Goats
- ✓Some Wild Species esp. Small Ruminants
- ✓Camels
- ✓Buffaloes
- ✓Cattle



Occasionally, often dead-end hosts and indicators of PPRV from the core of an episystem.

Unusual hosts

- ✓Yak
- ✓Pig
- ✓Dog
- ✓Asiatic lion
- ✓Biting midge



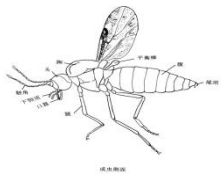


Table 1. Evidence of natural and experimental infection of PPRV in large ruminants, camels and unusual hosts.

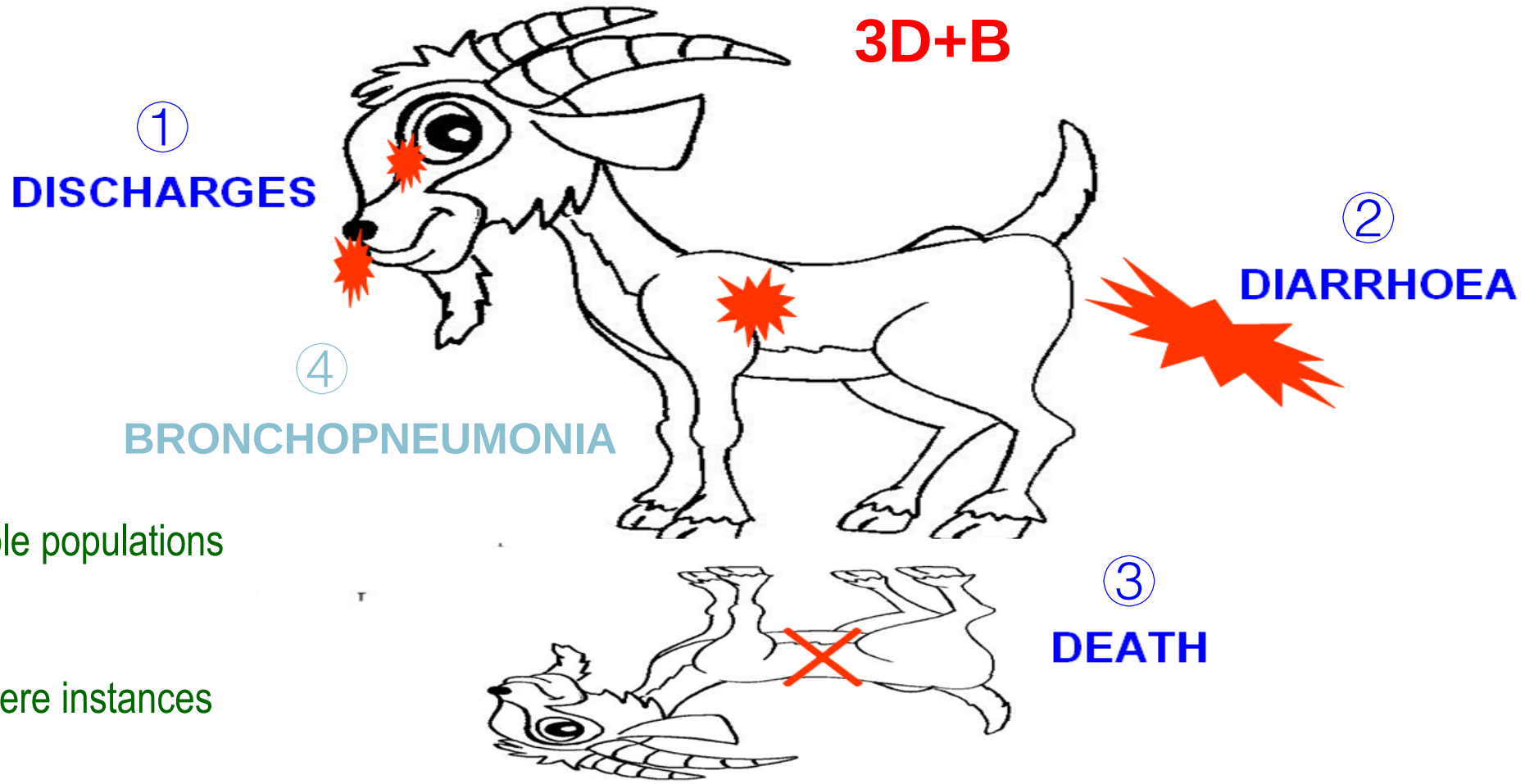
Host	Country	Year	References
Clinical infections of PPRV in large ruminants and camels			
Camel	Iran	2013	Zakian et al. (2016)
Camel	Sudan	2004-08, 2004	Kwiatek et al. (2011) and Khalafalla et al. (2010)
Camel	Ethiopia	1995-96, 2000-12	Roger et al. (2000) and Saeed et al. (2015)
Camel	Kenya	2016	Omani et al. (2019)
Water Buffalo	India	1995	Govindarajan et al. (1997)
Detection of antibodies in large ruminants and camels as a result of natural exposure to PPRV			
Cattle	Iran	Not available	Rasooli et al. (2019)
Cattle	Tanzania	2011, 2016	Herzog et al. (2019) and Lembo et al. (2013)
Camel	Kenya	Not available	Chemweno et al. (2019)
Cattle	Ethiopia	2005-2006, 2001	Agga et al. (2019) and Abraham et al. (2005)
Cattle, Yak	China	2016-17	Li et al. (2018)
Cattle	Sudan	2008-12, 2001, 2015-16, 2016-18	Haroun et al. (2002), Intisar et al. (2017), Ali et al. (2019), Hekal et al. (2019)
Camel	Sudan	2008-12, 2008, 2001, 2008-09	Haroun et al. (2002), Intisar et al. (2010, 2017), Saeed et al. (2010)
Cattle, Water Buffalo, Yak	Pakistan	2009, 2005-06, 2007, 2014	Khan et al. (2008), , Abubakar et al. (2017, 2019)
Camel	Nigeria	2011-03, 2012, 1995, 2012-13	Daneji et al. (1997), Bello (2013), El-Yuguda et al. (2013), Woma et al. (2015)
Camel	Libya	2014	El-Dakhly (2015)
Cattle, Water Buffalo	India	2011, 2009-10	Balamurugan, Krishnamoorthy et al. (2012), Balamurugan et al. (2014)
Cattle	Nigeria	2012-13	El-Yuguda et al. (2013)
Camel	Tanzania	2010	Swai et al. (2011)
Camel	India	Not available	Rajneesh and Tanwar (2011)
Cattle	Turkey	2009, Not available	Ozkul et al. (2002) andAlbayrak and Gur (2010)
Camel	Ethiopia	2001, 1995	Roger et al. (2000) and Abraham et al. (2005)
Cattle	Kazakhstan	1997-98	Lundervold et al. (2004)
Cattle	Bangladesh	1993, 1997-98	Anowar and Nadir (2004)
Cattle	Nigeria, Ghana	1993	Anderson and McKay (1994)
Camel	Egypt	Not available	Ismail et al. (1992)
Experimental infection of PPRV in camels			
Camel	Morocco	2018	Fakri et al. (2019)
Experimental infection of PPRV in large ruminants			
Cattle	Côte D'ivoire	2018	Couacy-Hymann et al. (2007)
Cattle	India	2013	Sen et al. (2014)
Experimental infection of PPRV in unusual hosts			
Pig	Germany	2015-2016	Schulz et al. (2018)
Pig	Nigeria	1978	Nawathe and Taylor (1979)
Evidence of PPRV nucleic acid in unusual hosts and vectors			
Biting midge	Turkey	2015	KU325483; https://www.ncbi.nlm.nih.gov/nuccore/KU325483. . . . . KU175171; https://www.ncbi.nlm.nih.gov/nuccore/KU175171. . . . .
Dog	India	2015	Ratta et al. (2016)
Asiatic lion	India	2007	Balamurugan et al. (2012)

Section 2: PPR Diagnostics

DIAGNOSIS OF PPR

- Clinical diagnosis
- Lab or pen-side diagnostic tools

CLINICAL DIAGNOSIS
D→D→B→D



Incubation period:

3-10d

21d, for the code

Morbidity:

90-100% , in susceptible populations

Mortality:

50 -100%, in more severe instances

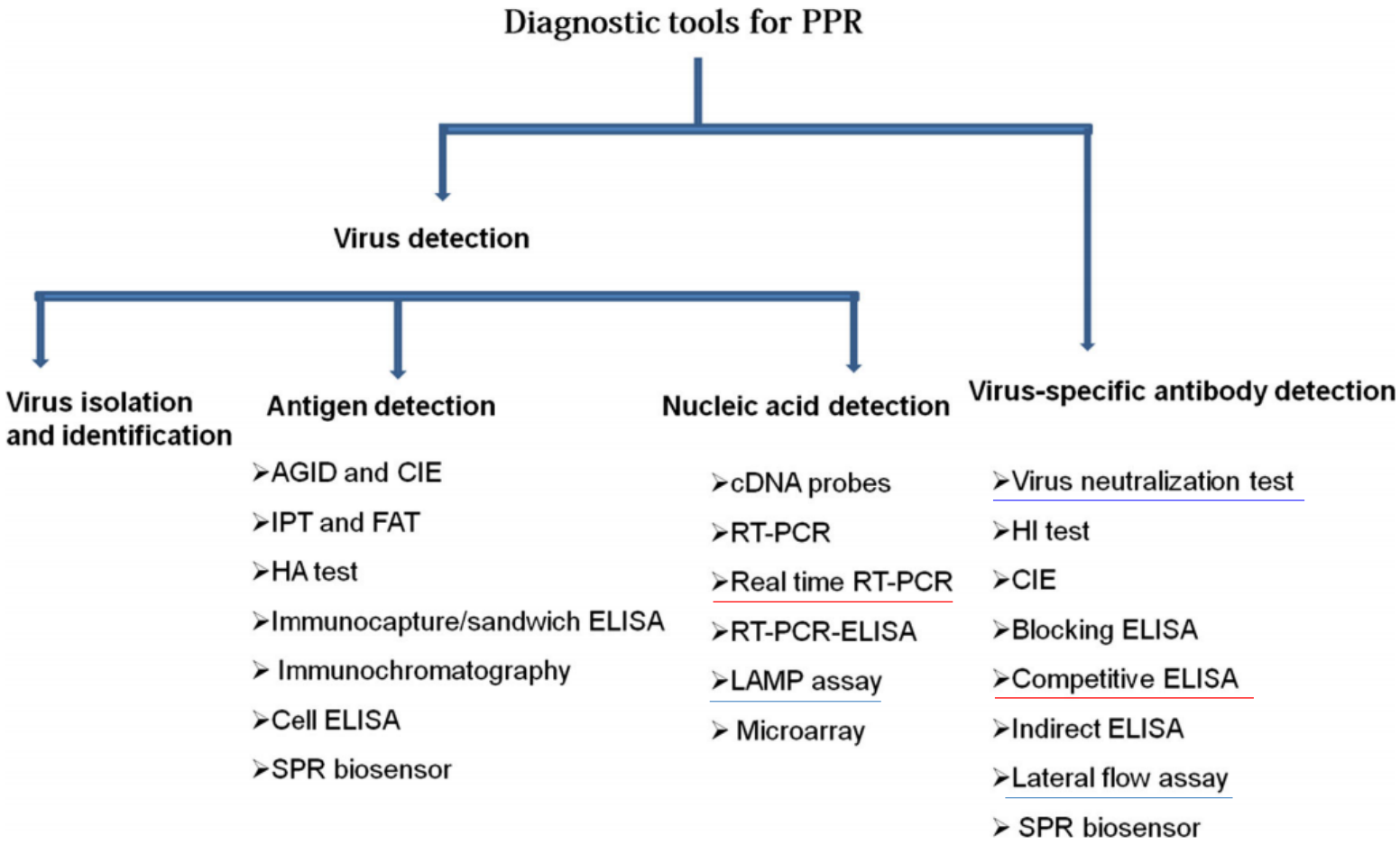
➢Xinjiang outbreaks during 2013-2014

- **Morbidity** (unvaccinated herds)
  Goat 25%-100% (varies with breeds, density and environment)
  Sheep 5-30%
- **Case Fatality**
  Goat 25-60%
  Sheep 5-15%

DIFFERENTIAL DIAGNOSIS

- BT
- FMD
- Orf
- CCPP
- HW
- Pasteurellosis
- Coccidiosis
- Mineral poisoning

DIAGNOSTIC TOOLS FOR PPR



可以凝集鸡、山羊和猪的红细胞

Ramasamy et al. Peste des petits ruminants diagnosis and diagnostic tools at a glance: perspectives on global control and eradication

PPR Diagnostic tools listed in the Manual

Sensitivity/Specificity/Repeatability/Reproducibility/Accessibility/Operability ,considered

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent[a]						
RT-PCR	–	++	++	+++	+	–
Real-time RT-PCR	–	++	+++	+++	+	–
Virus isolation in cell culture	–	–	–	++	–	–
Immunocapture ELISA		+	++	+++	+	–
Penside test (LFD)	–	–	++	++	–	–
AGID	–	–	+	+	–	–
Counter immune-electrophoresis	–	–	–	+	–	–
Detection of immune response						
Virus neutralisation	+++	–	–	++	++	++
Competitive ELISA	+++	–	+++	+	+++	+++
AGID	–	–	+	+	–	+
Counter immune-electrophoresis	–	–	–	+	–	–

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
RT-PCR = reverse-transcription polymerase chain reaction;
ELISA = enzyme-linked immunosorbent assay; LFD = lateral flow device; AGID = agar gel immunodiffusion.
[a]A combination of agent identification methods applied to the same clinical sample is recommended.

Recommendations for Virus Tests

1. Laboratory Tests

   Real time RT-PCR or

   Conventional RT-PCR + sequencing for new outbreaks

2. Field tests if Necessary and affordable

   Dipsticks/ LFD

3. Other tests may also be used based on your own conditions and the availability.

Recommendations for Antibody Test

- **Antibody Tests**
  - —cELISA
  - —VNT, Using known virus to test neutralizing antibody in serum
  - —LFD field rapid detection for antibody

- **Applications**
  - —Diagnosis in unvaccinated herds, especially at the late stage of an outbreak, e.g. more than 10 days after the infection.
  - —Post Vaccination Evaluation (PVE) for disease control and status recognition

PPRV antibody positive starts from 7-11 days PI

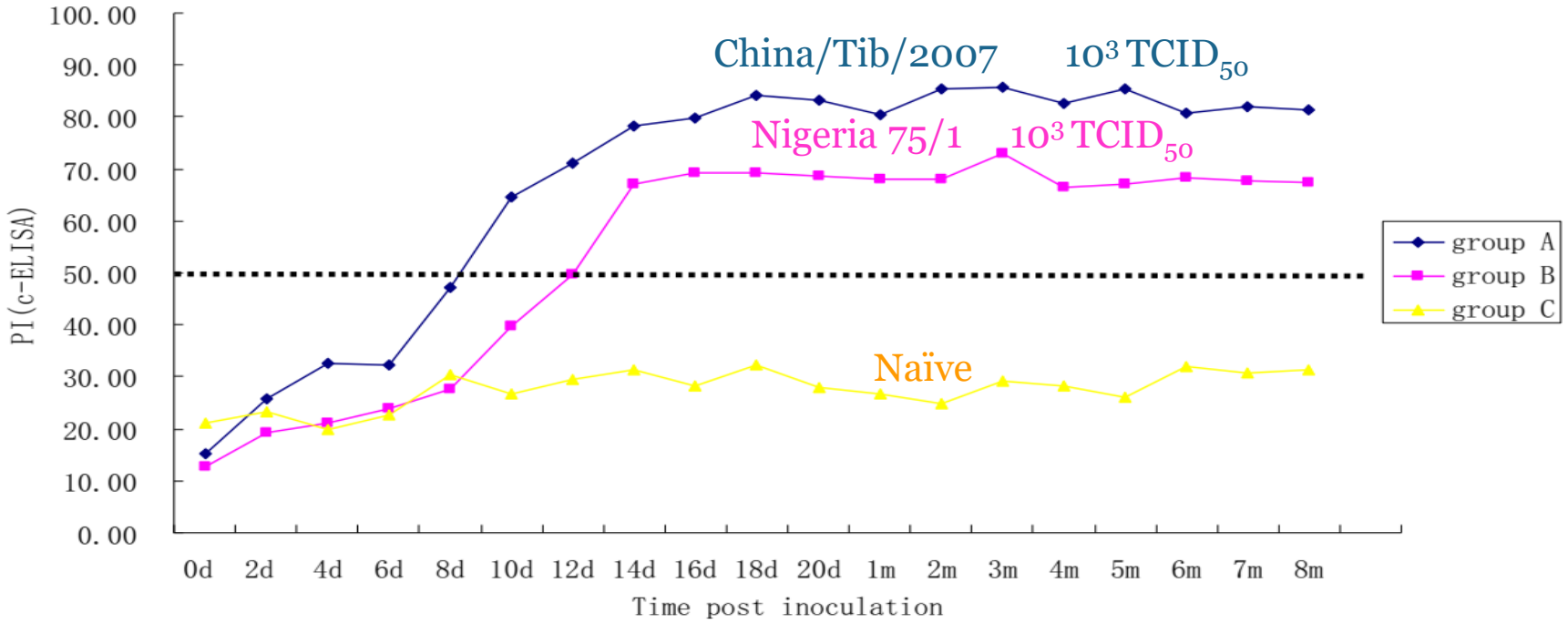


图3-7 c-ELISA检测不同组间的抗体水平

Fig.3-7. Anbibody response against PPRV using c-ELISA in different groups of goats
The dotted line represents the negative/positive cut-off value (50% inhibition).

Liu and Wang et al　local dairy goats

For other information on PPR diagnosis , you are welcome to visit our website:

https//www.ppr-labs-oie-network.org

Section 3: The Role of PPR RL in Support of Eradication through Episystem Approach

3.1 Training

- **Clinical Diagnosis, to find suspects**

  Personnel working with susceptible animals, such as

  Vets, CAHW, Keepers, Traders, Slaughterers, Wildlife Workers, etc.

- **Laboratory Diagnosis, to confirm an infection**

  Lab staff engaged in PPR diagnosis

- **Training Must be Enhanced in Episystems**

3.2 Validation of an assay: Principles and Methods

Chapter 1.1.6, WOAH Mannual

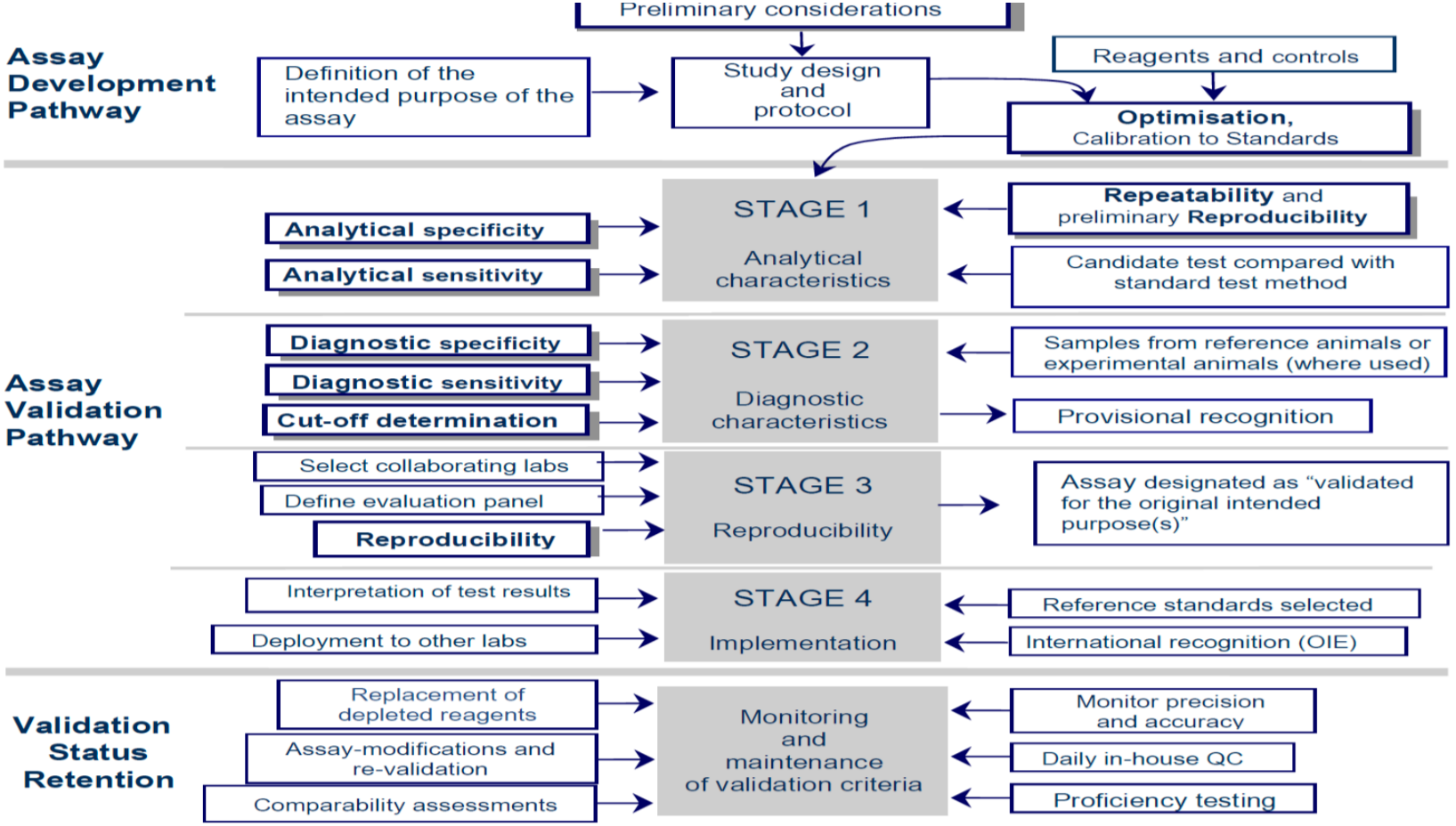


Figure 1. *The assay development and validation pathways with assay validation criteria highlighted in bold typescript within shadowed boxes.*

3.3 Proficiency Test among Laboratories
(WOAH Validation standard, section B.5.1, Chapter 2.2.6)

- **Organized by RL or the authority**
- **Double blind**
- **Identical protocols, reagents and controls**
- **Same panel and volume, but marked with different numbers among labs.**
- **Samples panel: at least 5 besides the controls**
  - 2 high positive
  - 2 low positive
  - 1 negative


小反刍兽疫病毒阻断 ELISA 抗体检测试剂盒说明书

兽用

【兽药名称】
通用名称：小反刍兽疫病毒阻断 ELISA 抗体检测试剂盒
商品名称：无
英文名称：Blocking ELISA Kit for Detecting Antibody of Peste des Petits Ruminants Virus
汉语拼音：Xiaochuchoushouyi Bingdu Jingzheng ELISA Kangti Jiance Shijihe

【主要成分与含量】

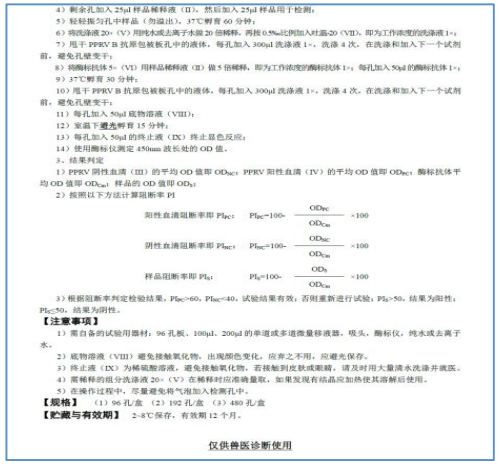
编号	试剂盒组分	数量 1P	数量 2P	数量 5P	用法
PPRV B-I	PPRV B 抗原包被板	96 孔板×1 块	96 孔板×2 块	96 孔板×5 块	直接使用
PPRV B-II	样品稀释液	7ml×1 瓶	14ml×1 瓶	35ml×1 瓶	直接使用
PPRV B-III	PPRV 阴性血清	100μl×1 管	200μl×1 管	500μl×1 管	直接使用
PPRV B-IV	PPRV 阳性血清	100μl×1 管	200μl×1 管	500μl×1 管	直接使用
PPRV B-V	洗涤液 20×	12ml×1 瓶	24ml×1 瓶	60ml×1 瓶	20 倍稀释后使用
PPRV B-VI	酶标抗体 5×	1ml×1 管	1ml×2 管	5ml×1 瓶	5 倍稀释后使用
PPRV B-VII	吐温-20	120μl×1 管	240μl×1 管	600μl×1 管	直接使用
PPRV B-VIII	底物溶液	5ml×1 瓶	10ml×1 瓶	25ml×1 瓶	直接使用
PPRV B-IX	终止液	5ml×1 瓶	10ml×1 瓶	25ml×1 瓶	直接使用

【作用与用途】用于检测绵羊、山羊血清或血浆中小反刍兽疫病毒（PPRV）抗体。

【用法与判定】
1、实验准备：试剂盒各个组分在使用前均须恢复至室温（20-25℃），加液前充分摇匀。
2、操作步骤
PPRV B 抗原包被板（I）上 PPRV 阴性血清（III）、PPRV 阳性血清（IV）、样品稀释液（II）和样品的分布如图 N、P、Cm 和 S 所示

	1	2	3	4	5	6	7	8	9	10	11	12
A	N	S5										
B	N											
C	P											
D	P											
E	S1											
F	S2											
G	S3											
H	S4											

1）在 A1 和 B1 孔加入 PPRV 阴性血清（III）各 50μl；
2）在 C1 和 D1 孔加入 PPRV 阳性血清（IV）各 50μl；
3）在 E1 和 F1 孔加入样品稀释液（II）各 50μl；


4）剩余孔加入 25μl 样品稀释液（II），然后加入 25μl 样品用于检测；
5）轻轻振匀孔中样品（勿溢出），37℃孵育 60 分钟；
6）将洗涤液 20×（V）用纯水或去离子水做 20 倍稀释，再按 0.5%比例加入吐温-20（VII），即为工作浓度的洗涤液 1×；
7）甩干 PPRV B 抗原包被板孔中的液体，每孔加入 300μl 洗涤液 1×，洗涤 4 次，在洗涤和加入下一个试剂前，避免孔壁变干；
8）将酶标抗体 5×（VI）用样品稀释液（II）做 5 倍稀释，即为工作浓度的酶标抗体 1×；每孔加入 50μl 的酶标抗体 1×；
9）37℃孵育 30 分钟；
10）甩干 PPRV B 抗原包被板孔中的液体，每孔加入 300μl 洗涤液 1×，洗涤 4 次，在洗涤和加入下一个试剂前，避免孔壁变干；
11）每孔加入 50μl 底物溶液（VIII）；
12）室温下避光孵育 15 分钟；
13）每孔加入 50μl 的终止液（IX）终止显色反应；
14）使用酶标仪测定 450nm 波长处的 OD 值。
3、结果判定
1）PPRV 阴性血清（III）的平均 OD 值即 OD_{NC}；PPRV 阳性血清（IV）的平均 OD 值即 OD_{PC}；酶标抗体平均 OD 值即 OD_{Cm}；样品的 OD 值即 OD_S；
2）按照以下方法计算阻断率 PI

阳性血清阻断率即 PI_{PC}：$PI_{PC}=100-\frac{OD_{PC}}{OD_{Cm}}\times 100$

阴性血清阻断率即 PI_{NC}：$PI_{NC}=100-\frac{OD_{NC}}{OD_{Cm}}\times 100$

样品阻断率即 PI_S：$PI_S=100-\frac{OD_S}{OD_{Cm}}\times 100$

3）根据阻断率判定检验结果，$PI_{NC}>60$，$PI_{PC}<40$，试验结果有效；否则重新进行试验；$PI_S>50$，结果为阳性；$PI_S\leq 50$，结果为阴性。

【注意事项】
1）需自备的试验用器材：96 孔板、100μl、200μl 的单道或多道微量移液器，吸头，酶标仪，纯水或去离子水。
2）底物溶液（VIII）避免接触氧化物，出现颜色变化，应弃之不用，应避光保存。
3）终止液（IX）为稀硫酸溶液，避免接触氧化物，若接触到皮肤或眼睛，请及时用大量清水洗涤并就医。
4）需稀释的组分洗涤液 20×（V）在稀释时应准确量取，如果发现有结晶应加热使其溶解后使用。
5）在操作过程中，尽量避免将气泡加入检测孔中。

【规格】（1）96 孔/盒（2）192 孔/盒（3）480 孔/盒

【贮藏与有效期】2-8℃保存，有效期 12 个月。

仅供兽医诊断使用

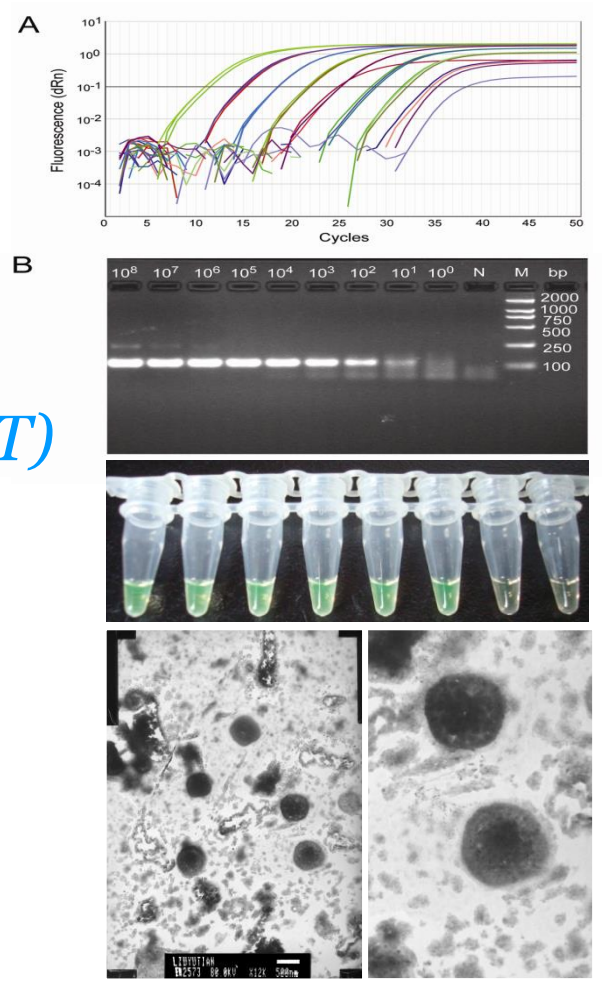
3.4 Confirmation of Infection

➢ First diagnosis

- *RT-PCR and partial sequencing*
- *Genome sequencing*
- *Virus isolation and identification (including NT)*
- *Antibody positive in unvaccinated animals*

➢ Following diagnosis

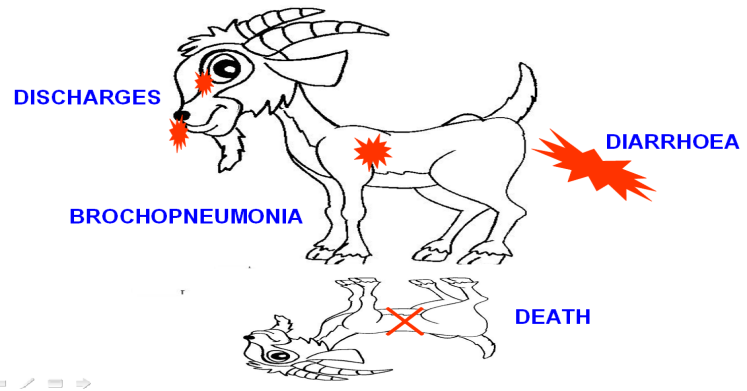
- *RT-PCR or Real time PCR as recommended*
- *Antibody positive in unvaccinated animals*
- *Sequencing and molecular analysis*

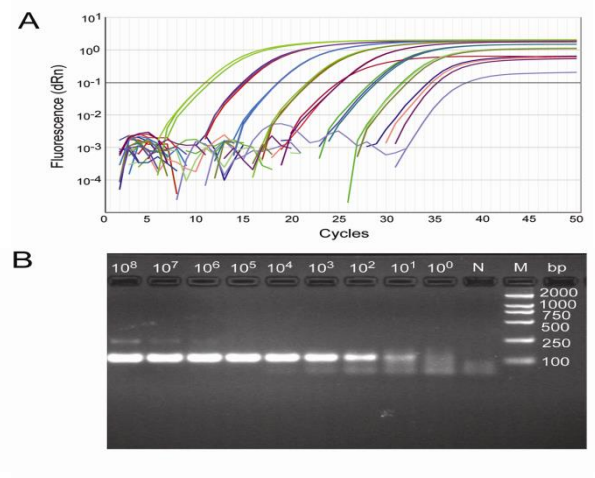


➢ Confirm an outbreak

- **Clinical Signs + Laboratory Positive**

- *Any positive resulted from vaccination should be ruled out.*





3.5 Supports to Surveillance and PVE

- **Assistance to design, implement and analysis, to**
  - **find spatial, temporal and herds distribution of PPR**
  - **trace outbreaks**
  - **identify Episystems for more targeted controls**
  - **know the exact coverage of vaccination the herds immunity and predict risks.**
- **Others as Dr.Guitian Javier presented**

3.6 Molecular Epidemiology Analysis

- Accumulate molecular data of PPRV in the country, region or even the globe
- Trace the source of PPRV in outbreaks from the molecular perspective
- Identify episystems in more intensive way through cluster analysis
- More details will be presented by Dr.Arnaud Bataille

SUMMARY

The Role of PPR RL
in Support of Eradication through Episystem Approach

- **Training: Clinical and Lab diagnostics, etc.**
- **Validation of Diagnostic Assay**
- **Proficiency Test**
- **Confirmation of Infection**
- **Supports to Surveillance and PVE** (Guitian Javier, FAO)
- **Molecular Epidemiolgy Analysis** (Arnaud Bataille,CIRAD)
- **Other Activities upon requests** (to be discussed)

Thank you for your attention