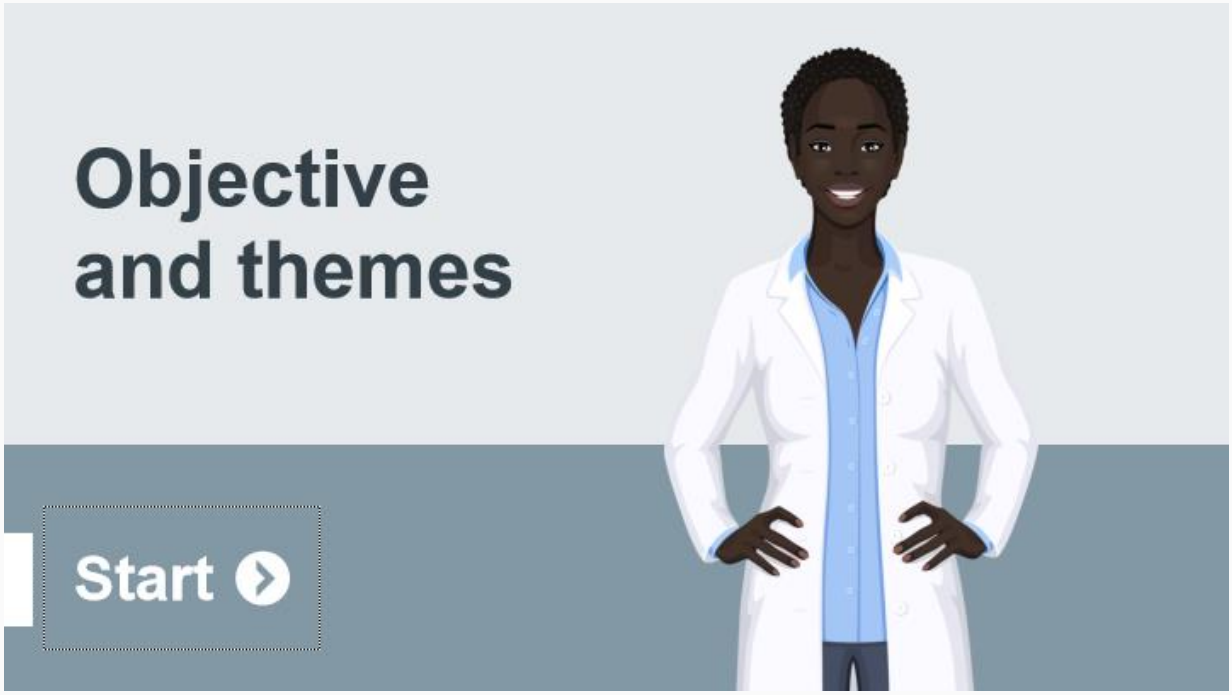
Rift Valley Fever - 5. Diagnosis

Objective and themes



Clinical and histopathological diagnosis

Rift Valley Fever must be suspected in domestic ruminants after observing during the mosquito season **abortions in several animals, high mortality** and pictures of **hepatic necrosis**.

Suspicion is strengthened:

- following the importation of animals from endemic areas,
- in the event of simultaneous flu-like symptoms in farm worker,
- following heavy rainfall and flooding.

During epidemics, abortion storm and cases of the disease in animals and humans are simultaneously observed.

The disease is characterized by severe hepatitis associated with abomasal haemorrhages in young animals or jaundice in adults.

Histologically, the most frequent picture is characterized by widespread hepatic necrosis.

Mortality in lambs

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Collecting samples

The samples to be sent to the laboratory for virological confirmation are:

- blood with EDTA, collected during the febrile phase of the disease;
- liver, spleen, lymph nodes;
- organs/ brain from aborted foetuses.

The samples should be kept on ice (**0-4°C**) during transport. If transport lasts for more than **24 hours**, samples should be stored in **glycerol-saline.**

Whole blood samples should also be taken, for serology.

Under **field conditions**, extreme care should be taken and protective clothing including gloves, mask and goggles should be worn during sampling.


BD Vacutainer Systems

While collecting samples it is important to collect all the relevant data:

- sampling site with reference map and complete address;
- name of owner, address for correspondence, telephone, etc. ;
- farm/herd/breed/infected type, group size and age;
- data of first case/sampling date;
- age of groups of healthy animals/survivors which have not aborted;
- complete clinical history;
- presence/absence of fever in humans;
- basic ecological characteristics of the infected area.

Complete the following sentences:

The samples to be sent to the laboratory for virological confirmation should include ..... collected during the febrile phase of the disease; liver, spleen, lymph nodes; organs/ brain from aborted foetuses.

The samples should be kept on ..... during transport.

When collecting the samples, it is important to collect data on the age of groups

of healthy animals/survivors which have not .....

blood with EDTA ice aborted

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Direct laboratory diagnosis

Virus isolation

The virus can be isolated more easily from **blood** taken during the febrile phase; from the **liver, spleen and brain** of dead animals and **aborted foetuses.**

Isolation can be carried out on:

- new-born mice;
- cell cultures (vERO, BHK_{21}, CER).

The virus causes a **cytopathic effect** with **destruction of the monolayer** in 3 days.

Identification may be carried out by direct immunofluorescence **after 18-24 hours** revealing **cytoplasmic inclusion bodies.**

Molecular biology

A rapid diagnosis may be carried out by **Reverse-Transcription PCR (RT-PCR).** The use of this technique followed by the sequencing of the amplified material is an excellent instrument for molecular epidemiology.

Another method is **cryostat sections** of tissues fixed in formalin and stained with immunohistochemical methods.

The **manipulation of virus alive can be performed only in laboratories** with BSL3 or BSL4 containment levels.


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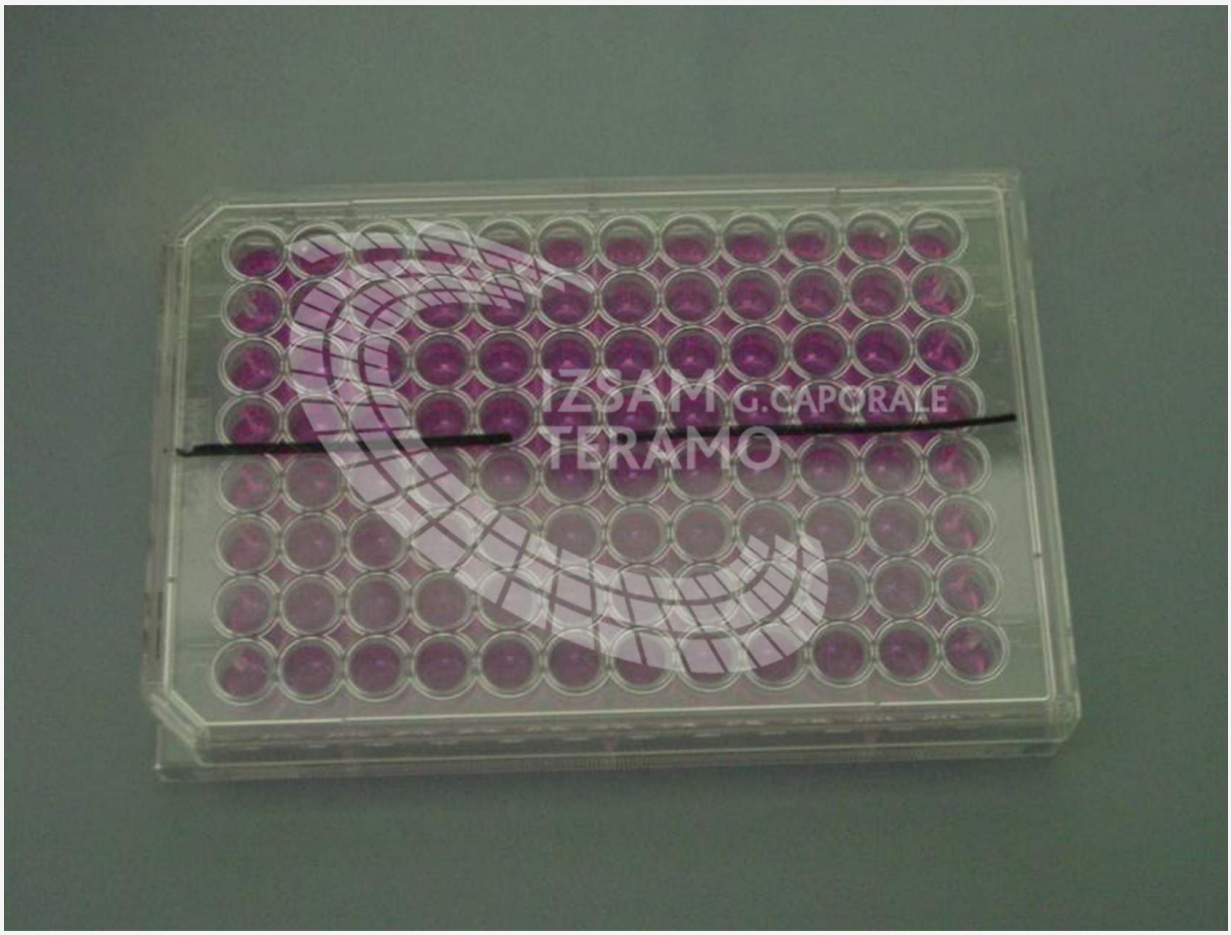
Indirect laboratory diagnosis

The **Plaque reduction neutralisation test (PRNT)** test is used for **serological diagnosis, and is highly specific.**

In **domestic ruminants** the antibody response may be revealed by PRNT starting from the 7th day after appearance of the initial signs and reaches maximum levels between the 15th and 21st day. The presence of neutralizing antibodies can be corroborated even many years after the infection, and probably for the entire productive life of the animal.

The Smithburn neurotropic mouse brain strain of highly attenuated RVFV or any other, preferably attenuated, RVFV, is used as challenge virus.



ELISA tests

Advantage of **ELISA** assays: they do not require the use of live virus, they can be used for the diagnosis in disease-free areas and can they be performed in normal diagnostic laboratories.

A number of **ELISAs** using different formats are commercially available. Both IgG and IgM ELISAs are available for most species. IgM-capture ELISA allows diagnosis of recent infections. In fact, usually IgM are no more detectable after 2-4 months from the infection.

Table 4: Details of the commercially available (*) or in-house developed ELISA tests

Name (manufacturer)	Format	Antigen	Tested species	Validation data	References
ID Screen® Rift Valley Fever Competition Multi-species (ID Vet)*	Competitive	Np rec (E. coli)	Multiple species, including ruminants, camels, horses, dogs and others	Sp%: 100 (CI 95%: 99.58–100%), n = 920 (bovine, ovine, caprine, horses, dogs, cats, human) Se%: 100 (CI 95%: 91.24–100%), n = 40 (bovine from Djibouti and Mayotte collected in 2008; 18 tested in VN)	El Mamy et al. (2011) and Comtet et al. (2010)
ID Screen® Rift Valley Fever IgM Capture (ID Vet)*	IgM capture	Np rec	Domestic ruminants (Anti-bovine-ovine-caprine IgM antibody) Springbok (*Antidorcas marsupialis*)	Not provided by manufacturer	
RVF recN IgG Indirect ELISA (BDSL)**	Indirect	Np rec (E. coli)	Human and livestock		Jansen van Vuren et al. (2007)
RVF Inhibition ELISA (BDSL)**	Inhibition	RVFV inac	Human, domestic ruminants, buffalo, camel	Sp%: 99.47 (humans), 99.52 (cattle), 99.65 (goats), 99.29 (sheep), 99.51 (buffaloes), 100 (camels) Se%: 99.47 (humans), 100 (cattle), 99.56 (goats), 100 (sheep), 100 (buffalo), 100 (camel)	Paweska et al. (2005)
RVF IgM ELISA (BDSL)**	IgM capture	RVFV inac	Domestic ruminants	Sp%: 98.7(sheep) 99.7 (goats) 100 (cattle)	Paweska et al. (2003)
INgezim FVR Compact R.13 FVR.K3 (Ingenasa)*	Competitive	Np rec	Domestic ruminants	Sp%:99 (n. 1526 cattle, sheep, goats) (n.1014 deer, ibex, mouflons, fallow deer, alpacas and zebra) Se%:97 (31 sheep experimentally infected)	
INgezim FVR IgM R.13.FVR.K2-(Ingenasa)*	IgM capture	Np rec	Domestic ruminants	Sp%: 99.3 Se%: 95.7 1589 ovine, caprine and bovine sera (experimentally infected and vaccinated animals. The negative samples corresponded to different RVFV-free areas in Spain)	
	Indirect	Np rec (baculovirus)	Sheep, cattle	Sp%: 97 (sheep) to 100 (cattle) Se%: 100 (vs. PRNT in sheep and cattle experimentally infected)	Faburay et al. (2019)
	Indirect	Gn rec (E. coli)	Small ruminants	Sp%: 95.6 Se%: 94.6 (n. 1952 sheep and goat sera from Mozambique, Senegal, Uganda and Yemen)	Jäckel et al. (2013)
	Double Ag ELISA (IgM and IgG detection)	Refer to William (2011)	Cattle and sheep	Sp%: 100 Se%: 98.4 (412 sheep and 121 cattle)	Ellis et al. (2014)
	IgM capture	Np rec (E. coli)	Small ruminants and cattle	–	Williams et al. (2011)
	Competitive	Np rec (E. coli/ Mab)	Cattle and goat	Sp%: 99.7 Se%: 94.7 (n. 105 blood samples collected at intervals from experimental infection of 2 cattle and 5 goats)	Kim et al. (2012)
	Indirect	Np rec + NSs rec	Human, goats	–	McElroy et al. (2009)
	Indirect with IgG and IgM conjugates	Np rec (E. coli)	Sheep, goat, cattle	Sp%: 99.5–100 (goats), 100 (sheep), 98.3 (cattle) Se%: 99.4–100 (goats), 100 (sheep), 100 (cattle)	Fafetine et al. (2007)

*: Np rec, recombinant nucleocapsid protein; Gn rec, recombinant glycoprotein Gn; NSs rec, recombinant Non-structural proteins.
**: Not commercially available at the present.

Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Depner K, Drewe JA, Garin-Bastuji B, Rojas JLG, Schmidt CG, Michel V, Chueca M_AM, Roberts HC, Sihvonen LH, Stahl K, Calvo AV, Viltrop A, Winckler C, Bett B, Cetre-Sossah C, Chevalier V, Devos C, Gubbins S, Monaco F, Sotiria-Eleni A, Broglia A, Abrahantes JC, Dhollander S, Van Der Stede Y and Zancanaro G, 2020. Rift Valley Fever – epidemiological update and risk of introduction into Europe. EFSA Journal 2020;18(3):6041, 72 pp. https://doi.org/10.2903/j.efsa.2020.6041

Test your knowledge:

The virus grown on cell cultures (TRUE, BHK_{21}, CER) and it does not induce a cytopathic effect.

- ◯ True
- ◯ False

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In domestic ruminants, the antibody response can be demonstrated with plaque reduction neutralisation test from the 7th day after appearance of the first signs.

- ◯ True
- ◯ False

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ELISA test can only detect IgG antibodies against RVFV.

- ◯ True
- ◯ False

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Differential diagnosis

Diagnosis of RVF must consider also the following diseases:

Nairobi sheep desease

Like RVF, in **small ruminants** it causes:

- abortion,
- high mortality,
- gastroenteritis.

Differently from RVF:

- the pathogenicity is lower in neonates,
- clinical signs and abortions are more sporadic,
- mortality is higher in adults,

- the carcasses show haemorrhages without signs of hepatitis,
- there is a different fever* pattern.

***The fever trend in typically biphasic in Rift Valley Fever, while it is monophasic in the Nairobi Sheep Disease.**

Heartwater

Characterised by:

- lymphadenopathy,
- central nervous system involvement,
- respiratory tract involvement (pulmonary or pericardial oedema).

Post mortem findings: the absence of hepatitis and the presence of large volumes of exudate in the chest and abdominal cavity enable differentiation of the two diseases.

Ephemeral fever	This disease only affects cattle and causes nasal discharge, ocular discharge and agalactia, very similar to the signs of RVF but the fever is more intense. Muscular weakness and the
Wesselbron Disease (WSL)	The clinical picture of this disease is similar to and occurs in the same conditions as RVF. Both RVF and WSL viruses can cause mortality in lambs, kids and calves and abortion in ewes, but RVF
Peste des petits ruminants (PPR)	Characterised by fever and higher mortality in young animals. PPR causes oral erosion and severe respiratory

distress, which are not present in cases of RVF.

Toxoplasmosis, Leptospirosis, Brucellosis, Q Fever, Salmonellosis

These diseases have similar signs of RVF but have different mortalities and temporal and geographical distribution. They are not generally associated with heavy rainfall and

In the presence of **haemorrhagic syndromes** and **hepatic and haemorrhagic lesions** it is necessary to distinguish RVF from copper poisoning, pasteurellosis and Salmonellosis.

In humans, **RVF** is mainly confused with **flu-like** syndromes. Other diseases to be considered include:

- Q fever,
- Brucellosis,
- Haemorrhagic fevers.

Which of these diseases does not need to be considered in differential diagnosis against RVF?

- Q Fever
- Ephemeral Fever
- Foot and Mouth Disease
- Toxoplasmosis
- Nairobi Sheep Disease

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Summary