Public Health England

THE Pirbright INSTITUTE

Efficacy study for a novel vaccine against Crimean-Congo Haemorrhagic fever

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IVVN-EuFMD Vaccine field trial workshop

BBSRC bioscience for the future

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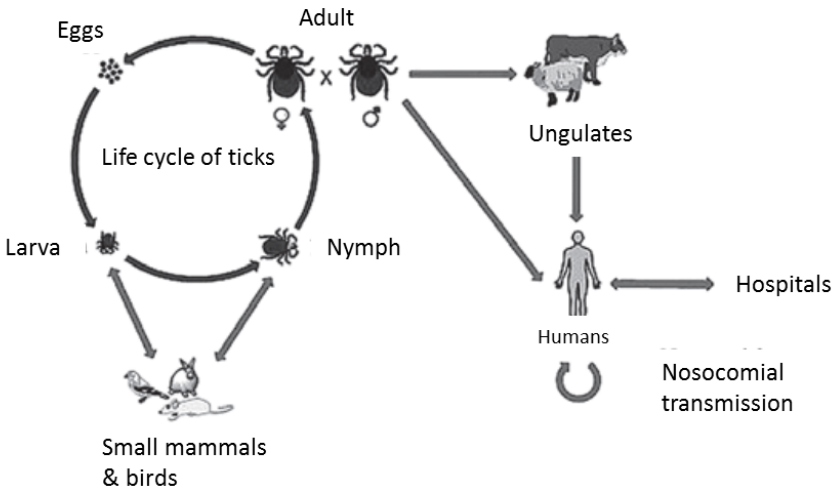
Background

- Crimean-Congo haemorrhagic fever virus (CCHFV) is a tick borne human pathogen of the utmost seriousness being both fast acting and with high mortality rates in humans.
- CCHFV belongs to the family *Bunyaviridae,* genus *Nairovirus*
- CCHFV is transmitted most commonly by *Hyalomma marginatum* and *Rhipicephalus sanguineus* ticks and contact with infected livestock and their body fluids.
- Livestock get infected but does not show clinical signs.



From: Aslam *et al 2016*

Background

- No commercial vaccine exists. A vaccine based on CCHFV, amplified in mouse brain and inactivated by chloroform treatment has been used (in humans) in Eastern Europe.

- PHE developed a pox-vectored vaccine based on **Modified Vaccinia virus Ankara (MVA)** encoding the envelope glycoprotein (GP) spikes of CCHFV that are the major targets of a protective neutralizing antibody response.

- This vaccine induces protection in interferon deficient mice (the only experimental animal infection model available).

Project objectives

- Develop a vaccine that can be used for sheep and non-human primates.
  - Demonstrate that the vaccine can block viremia and sero-conversion to viral proteins not expressed in the vaccine to differentiate between infected and vaccinated animals (DIVA).
- Assess whether the vaccine induces immune responses in sheep and non-human primates.
- Assess whether the vaccine prevents infection in sheep (to limit exposure of humans) after natural challenge in a field study in an endemic area, during periods of high levels of transmission in the tick season.
- Validate the vaccine platform for use in humans (combining results from studies in sheep, combined with immunogenicity studies in non-human primates).

Project objectives

Assess whether the vaccine prevents infection in sheep (to limit exposure of humans) after natural challenge in a field study in an endemic area, during periods of high levels of transmission in the tick season.

Previous to designing the vaccine trial:

- *Identify hot spot areas*
- *Estimate incidence in the non-vaccinated population*
- *Within and between cluster variance of incidence*
- *Assess feasibility and logistics*
- *Assess farmers willingness to participate in the vaccination trial.*

Information available

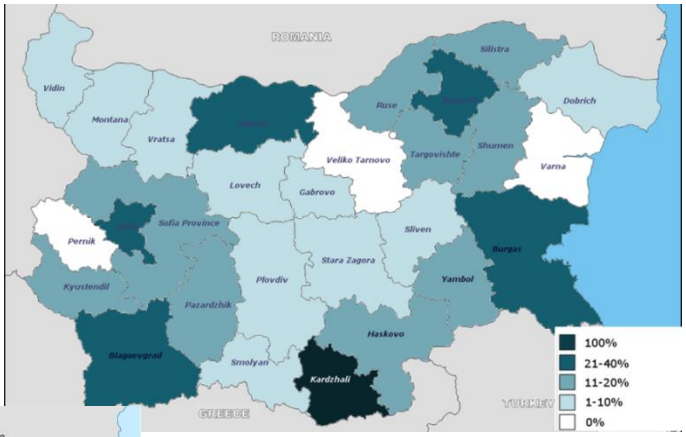
Previous estimations on the level of CCHF infection in domesticated livestock, only estimate the overall proportion of seropositive animals.

TABLE 1. RESULTS OF THE SEROEPIDEMIOLOGICAL STUDY IN ANIMALS (SHEEP, GOAT, CATTLE) OF BULGARIA (2011/2014)

Provinces	*No. of samples*	*Seroprevalence rates (%)*[a]
Blagoevgrad	72	38 (26–50)
Burgas	122	50 (41–59)
Dobritch	20	0 (0–17)
Gabrovo	15	13 (2–40)
Haskovo	85	34 (24–45)
Lovetch	75	0 (0–5)
Montana	6	0 (0–46)
Plovdiv	21	5 (0–24)
Shumen	30	0 (0–12)
Sliven	7	57 (18–90)
Yambol	166	24 (18–31)
Total	619	26 (23–30)

[a]95% confidence intervals shown in brackets.

From: *Mertens et al 2016*



Personal communication Iva Christova

From: *Panayotova et al 2016*

First field study

- Estimate CCHF farm sero-prevalence in young sheep in two Regions: Burgas and Kardzhali
- Estimate within-farm and between farm variation for CCHF infection in sheep and identify hot spot areas for sheep infection.
- Identify factors associated with higher risk of CCHF infection in sheep at farm level.

B=Burgas
K=Kardzhali

First field study

- Number of sheep to be sampled (in each region):

  Expected prevalence 50% (worse case scenario)

  95% confidence

  6% precision for an expected prevalence of

- Adjusted for:

  Intra-cluster (intra-farm) correlation coefficient (ICC) 0.12

  Number of animals to be sampled in each farm (m) 10

555 animals per region

600 animals per region → **60 farms**

First field study

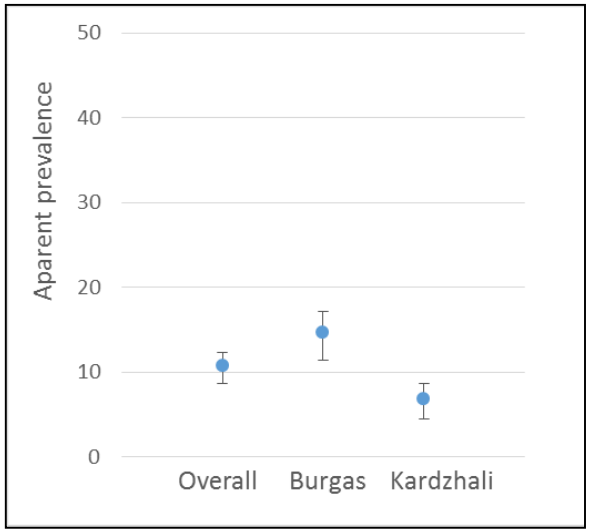
- 120 farms were randomly selected (60 in each Region)

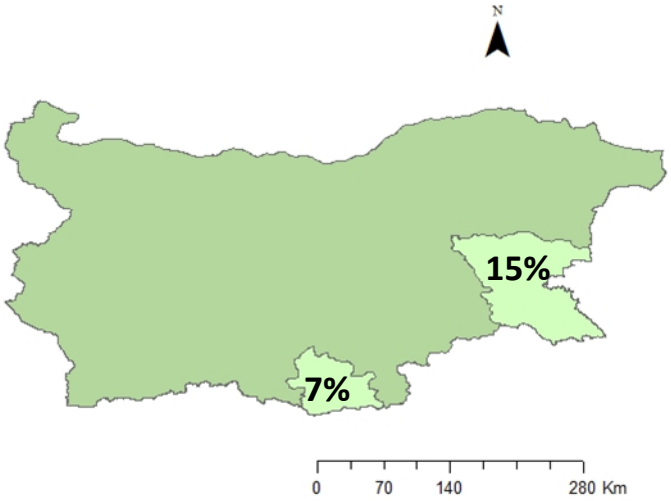
- In each selected farm:
  - 5 lambs (between 3 months and 12 months) and 5 sheep between 13 and 24 months were systematically selected – a blood sample and animal data was collected.
  - Farm characteristics & management practices were collected using Epicollect.

Results - First field study

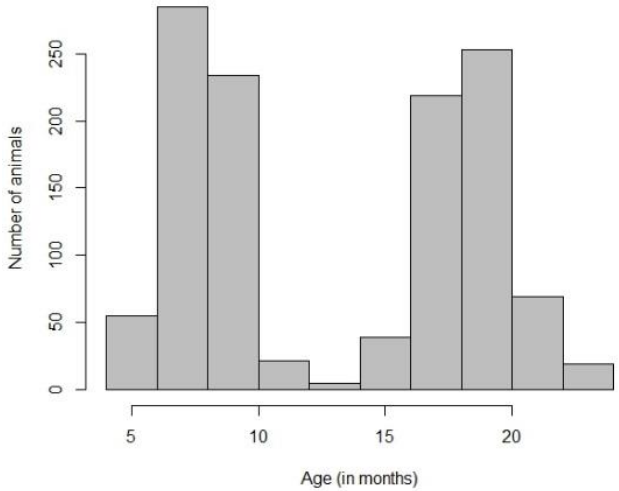
- Considering inconclusive samples as negative

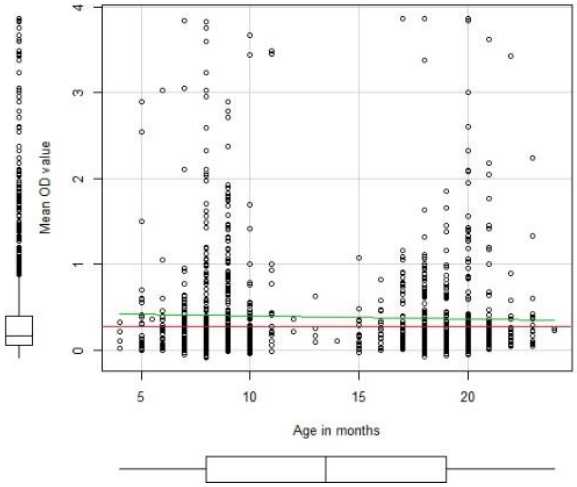
	ELISA	
	OD 0.5 cut-off % (95% CI)	OD 0.9 cut-off N (95% CI)
Overall	20.3 (18.0 – 22.7)	10.7 (9.1-12.7)
Burgas	26.0 (22.6 – 29.7)	14.6 (12.0-17.8)
Kardzhali	14.5 (11.8 – 17.6)	6.8 (5.0-9.2)





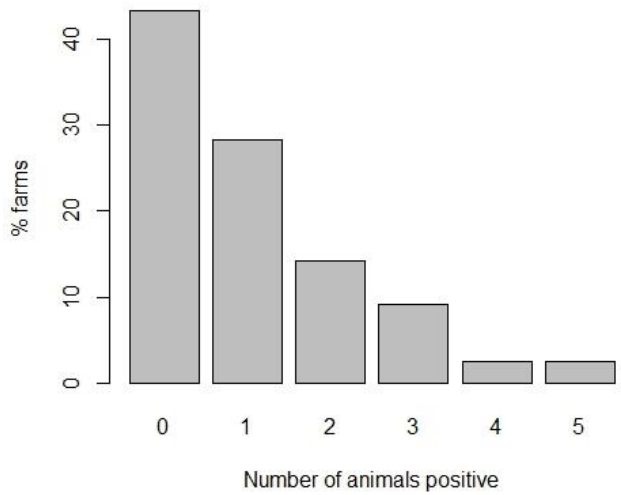
Results - First field study

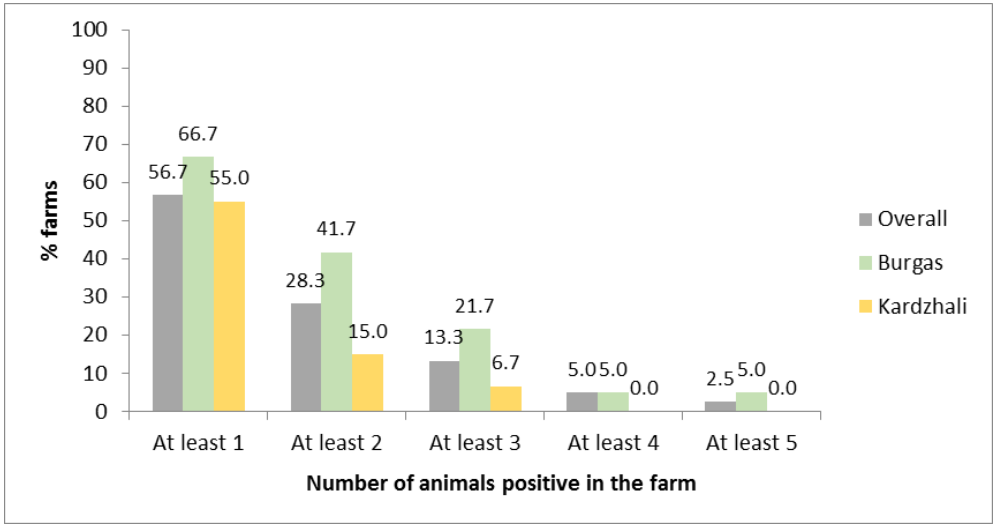


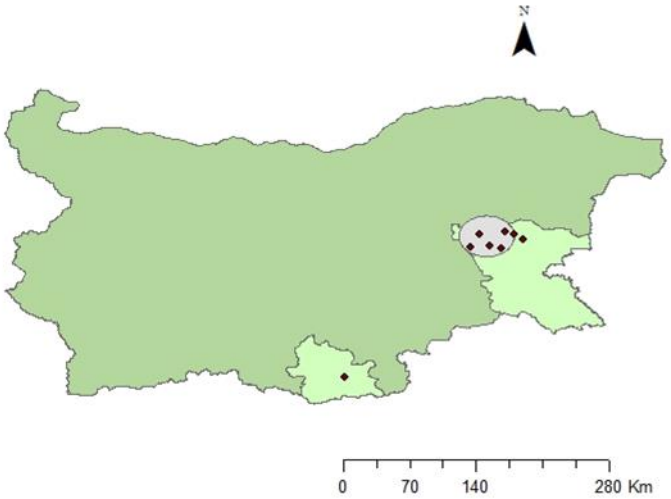


Age	No. negative (%)	No. negative (%)	OR (95% C.I.)	P value
13-24 months (young adults)	548 (90.7)	56 (9.3)	*reference*	
3-12 months (lambs)	523 (87.7)	73 (12.2)	1.40 (1.01-1.79)	0.08

Results - First field study





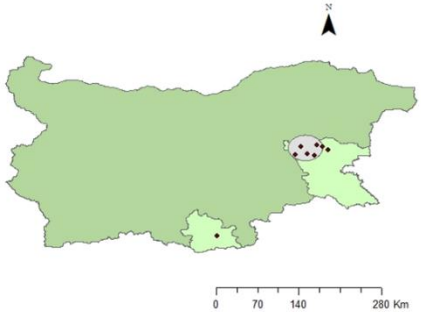


Follow up study

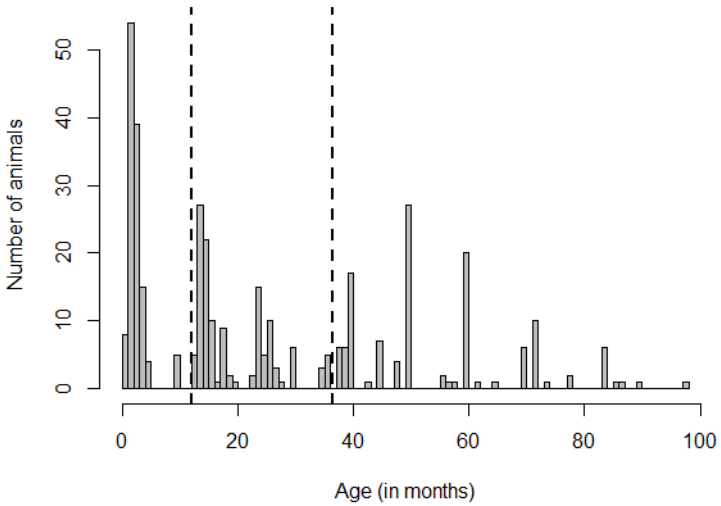
- Conducted in March 2018
  - Assess if maternal antibodies have weaned off in lambs by the beginning of March (same time of the year when the vaccine trial will take place).
  - Estimate within farm prevalence, stratified by age, in farms located in the hot spot area identified (North West Burgas).

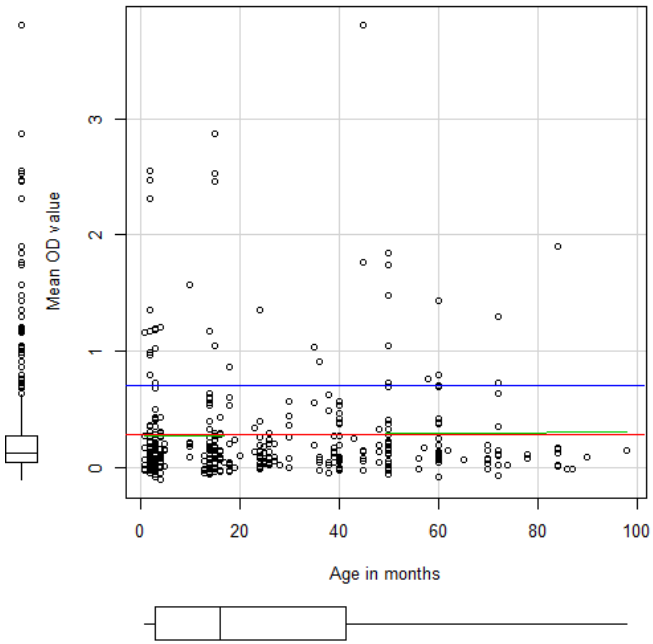
- Re-visit farms located in the cluster area (NW Burgas – Sungulare, Ruen and Karnobat Municipalities) that still operate plus other farms in the same area (n=25).
- Sample 15 animals and collect animal data.

Age	***Num. animals to be sampled***
3 – 12 months	5 animals
13 – 36 months (1-3 years)	5 animals
>36 months (>3years)	5 animals



Results – follow up study





375 animals sampled

- **125** animals were between **1 and 12** months (median 3 months)
- **127** animals between **13 and 36** months (median 16 months)
- **123** animals **>36 months** (median 50 months)

- Most animals sampled (98.1%) were born in the same farm

Results – follow up study

Status	Num. animals (%)
Positive	28 (7.5)
Negative	343 (91.5)
Inconclusive	4 (1.0)
	Apparent prevalence (95% C.I.)
Overall (n=375)	7.5 (5.1-10.7)
Lambs (1-12 months) (n= 125)	8.8 (5.0-15.6)
Adults (13-24 months) (n=94)	6.4 (2.6-13.9)
Adults (25-36 months) (n=33)	6.1 (1.1-21.6)
Adults (>36 months) (n=123)	7.6 (4.0-14.2)

Lessons learnt from field studies

Challenges

- Randomisation of farms and animals – difficult for field vets to get the importance of it.
- Sampling time was tight in the first field study, but not so much in the follow up when vets were more familiar.

Positive points

- Farmers were more familiar in the follow up study
- Very helpful to discuss unexpected results with field vets.
- Trust and positive farmers - vets relationship in the study area made things easier

Vaccine efficacy study

A multisite randomised two arms control trial will be conducted in the north western part of Burgas district, Bulgaria

The main aim is to determine the vaccine efficacy after natural challenge in sheep in a high-risk area and during periods of expected high levels of transmission.

A secondary objective is to assess how long humoral response (IgG antibodies) last and assess the difference between animals naturally exposed (placebo) and vaccinated.

Vaccine efficacy study

Sample size:

- There was no information available on vaccine efficacy in sheep. The only study available is in mice and reported 100% protection. **We considered three conservative estimates: 40%, 50% and 60%**
- Given differences on incidence and between farm variation obtained in both field studies, **we estimated the sample size using different parameters derived from each field study**

$$c = 1 + \left(Z_{\alpha/2} + Z_{\beta}\right)^2 \frac{\pi_0 \left(1 - \frac{\pi_0}{m} + \frac{\pi_1 (1 - \pi_1)}{m} + k^2(\pi_0^2 + \pi_1^2)\right.}{(\pi_0 - \pi_1)^2}$$

c = the required number of sites (farms)

π_1 and π_0 = the true proportions in the presence and absence of the intervention, respectively

m = is the number of sheep from each farm

k =is the between cluster coefficient of variation.

Vaccine efficacy study

Vaccine efficacy•	Incidence in non-vaccinated ◊	Total animals without adjusting for clustering	Between site (farm) variation	Num. of animals per site (farm)	Num. of farms (both arms)	Total animals vaccinated	Total animals placebo
40%	*28.6	416 (208 per arm)	0.016	10	44	220	220
				15	30	225	225
				20	24	240	240
	**16.9	796 (398 per arm)	0.021	10	82	410	410
				15	56	420	420
				20	24	420	420
	†13.3	1046 (523 per arm)	0.026	10	108	540	540
				15	72	540	540
				20	56	560	560
	‡7.5	1958 (979 per arm)	0.035	10	198	990	990
				15	134	1005	1005
				20	102	1020	1020
50%	*28.6	252 (126 per arm)	0.016	10	28	140	140
				15	20	150	150
				20	16	160	160
	**16.9	480 (240 per arm)	0.021	10	50	250	250
				15	34	255	255
				20	26	260	260
	†13.3	630 (315 per arm)	0.026	10	66	330	330
				15	44	330	330
				20	34	340	340
	‡7.5	1176 (588 per arm)	0.035	10	120	600	600
				15	82	615	615
				20	62	620	620
60%	*28.6	164 (82 per arm)	0.016	10	20	100	100
				15	14	105	105
				20	12	120	120
	**16.9	312 (156 per arm)	0.021	10	34	170	170
				15	24	180	180
				20	18	180	180
	†13.3	408 (204 per arm)	0.026	10	44	220	220
				15	30	225	225
				20	24	240	240
	‡7.5	764 (382 per arm)	0.035	10	80	400	400
				15	54	405	405
				20	42	420	420

Vaccine efficacy study

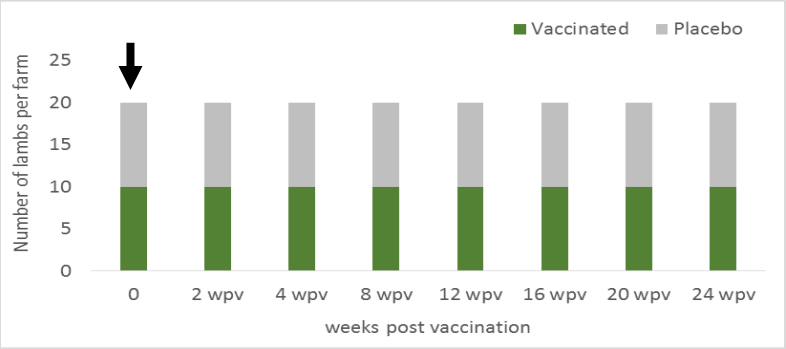
- 26 farms 520 animals (260 vaccinated 260 placebo)
  - 10% lost in follow up during 6 months → to 572 animals,
    - increased to **580 animals** to have equal number of animals per farm, giving a total number of **29 farms**
- In each site (farm)
  - 10 animals will receive the vaccine
  - 10 animals will receive placebo

34 farms have accepted to take part

- Farmers, field vets and lab people will be blinded (triple blinded)
- Personal information will be kept strictly confidential.
  - Farm and animals will be given a unique project number (individual ear tags).
- Farmers will be compensated

Vaccine efficacy study

- Animals will be followed up for 6 months.
  - Blood sample will be collected from each lamb taking part of the study before applying the vaccine or placebo and 2, 4, 8, 12, 16, 20 and 24 weeks after.



Vaccination day (day 0)	Weeks						
	2wpv	4wpv	8wpv	12wpv	16wpv	20wpv	24wpv
Between 5th and 10th March	16th to 24th March	30th March to 7th April	29th to 5th May	26th to 1st June	23rd to 30th June	21st July 28th July	18th to 25th August

Vaccine efficacy study

- Data to be collected:
  - Animal data (ear-tag number, live weight, rectal temperature, presence of ticks)
  - Health events since the previous visit will be collected for each animal.

Vaccine efficacy study

Animal allocated treatment (vaccine or placebo) will be disclosed to the farmers and field teams at the end of the study.

According to the Bulgarian regulation animals that received the vaccine cannot enter the food chain and therefore have to be culled at the end of the study and carcasses disposed accordingly.

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ALL FARMERS!

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Questions