Supplementary Materials:

Tab. S1 Overview of TBEV-RNA copies/500 µl homogenate (sample: size of a rice grain) as determined by RT-qPCR in the small intestine and cerebrum of mice subcutaneously infected with 10^3 TCID50/ml of TBEV Neudoerfl strain. M1-M3 were medium injected negative controls while M29 and M30 were intracerebral infected positive control mice. (dpi, day of euthanasia after TBEV-infection).

Tab. S2: Overview of TBEV-RNA copies/500 µl homogenate (sample: size of a rice grain) as determined by RT-qPCR in the small intestine, cerebrum and cerebellum of mice orally infected with 10^3 TCID50/ml of TBEV Neudoerfl strain and histological findings within the brain. M1-M3 were medium injected negative control mice. (dpi, day of euthanasia after TBEV-infection).

Fig S1 Clinical Score sheet used for TBEV murine infection model.

Fig. S2 Ratio (2delta-delta Cq) of Cq values of TBEV RNA copies/5 µl vs β-actin copies /5 µl of small intestine, spinal cord, cerebrum and cerebellum representing the relative expression differences over the course of infection. The spleen TBEV and β-actin RNA copies for each animal were used as reference organ.

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Animal ID	dpi	TBEV RNA copies in intestine	TBEV RNA copies in cerebrum
M4	0	Negative	Negative
M5	0	Negative	Negative
M6	0	Negative	Negative
M7	2	Negative	Negative
M8	2	Negative	Negative
M9	2	Negative	Negative
M10	4	$5.19 * 10^3$	Negative
M11	4	Negative	Negative
M12	4	$5.25 * 10^2$	Negative
M14	7	$1.06 * 10^4$	$6.17 * 10^4$
M15	7	$1.75* 10^7$	$4.63 * 10^6$
M28	7	$3.15 * 10^4$	$9.28 * 10^6$
M24	8	$1.52 * 10^6$	$1.59 * 10^9$
M25	8	$2.12 * 10^6$	$6.13 * 10^9$
M26	8	$1.23 * 10^6$	$2.71 * 10^8$
M18	9	$1.27 * 10^6$	$3.53 * 10^9$
M19	9	$1.00 * 10^6$	$1.74 * 10^9$
M17	10	$6.9 * 10^5$	$7.32 * 10^9$
M22	10	$7.08* 10^5$	$3.27 * 10^9$
M27	10	$9.22 * 10^5$	$6.89 * 10^8$
M21	11	$2.12 * 10^5$	$3.83 * 10^8$
M20	12	$3.65 * 10^5$	$1.08 * 10^9$
M23	12	$5.99 * 10^5$	$1.81* 10^9$
M16	14	Negative	Negative
M1	14	Negative	Negative
M2	14	Negative	Negative
M3	14	Negative	Negative
M29	6	$3.04 * 10^7$	$2.362 *10^{10}$
M30	6	$1.83 * 10^7$	$2,34 *10^{10}$

Tab. S2. Overview of TBEV-RNA copies/500 µl homogenate (sample: size of a rice grain) as determined by RT-qPCR in the small intestine, cerebrum and cerebellum of mice orally infected with 10^3 TCID50/ml of TBEV Neudoerfl strain and histological findings within the brain. M1-M3 were medium injected negative control mice. (dpi, day of euthanasia after TBEV-infection).

Animal ID	dpi	TBEV RNA copies in intestine	TBEV RNA copies in cerebrum	TBEV RNA copies in cerebellum	Histological findings within the brain
Mo4	0	Negative	Negative	Negative	Negative
Mo5	0	Negative	Negative	Negative	Negative
Mo6	0	Negative	Negative	Negative	Negative
Mo7	2	Negative	Negative	Negative	Negative
Mo8	2	Negative	Negative	Negative	Negative
Mo9	2	Negative	Negative	Negative	Negative
Mo10	4	Negative	Negative	Negative	Negative
Mo11	4	Negative	Negative	Negative	Negative
Mo12	4	Negative	Negative	Negative	Negative
Mo13	7	Negative	Negative	Negative	Negative
Mo14	7	Negative	Negative	Negative	Negative
Mo15	7	Negative	$6.37*10^3$	Negative	Negative
Mo16	10	Negative	$6.3*10^3$	$5.74*10^5$	Mild meningitis
Mo17	10	Negative	Negative	Negative	Negative
Mo20	10	Negative	Negative	$1.7*10^2$	Mild encephalitis
Mo18	14	Negative	Negative	Negative	Negative
Mo19	14	Negative	Negative	$4.68*10^4$	Negative
Mo21	14	Negative	Negative	Negative	Negative
Mo22	17	Negative	Negative	Negative	Negative
Mo23	17	Negative	Negative	Negative	Negative
Mo24	17	Negative	Negative	Negative	Negative
Mo25	21	Negative	Negative	Negative	Negative
Mo26	21	Negative	Negative	Negative	Negative
Mo27	21	Negative	Negative	Negative	Negative
Mo28	21	Negative	Negative	Negative	Negative
Mo1	21	Negative	Negative	Negative	Negative
Mo2	21	Negative	Negative	Negative	Negative
Mo3	21	Negative	Negative	Negative	Negative

Clinical Score Sheet for mice inoculated with tick-borne encephalitis virus (TBEV)

Table modified according to EC.Europe Directive 2010/63/EU (2012); (Foltz et al. 1999; OECD 2000; Pope et al. 2013; Morton and Griffiths, 1985; Visser, 1995; Conti et al., 2006; Haskins, 1992; Gärtner und Militzer, 1993; Becker, 2017)

Clinical score parameter severity of disorder in mice		Score
Bodyweight		
<5% weight loss		0
5-10% weight loss		1
11-20 % weight loss		2
acute weight loss > 20% in comparison to inital weight (≤24h)	*	HEP
weight loss > 25% in comparison to initial weight	*	HEP
Cardiovascular system		
normal		0
pinched skin/mild enophthalmia (sunken eyes)/mild dehydration		1
moderate to severe dehydration/moderate to severe ophtalmia (sunken eyes)		HEP
animal cold, legs and abdominal skin dark blueish		HEP
Coat/Skin Condition		
trimmed		0
Coat slightly unkempt, mild small skin irritation		1
Slight piloerection or small lesions, moderate small skin irritation or inflammation		2
Marked piloerection or moderate (scale) lesions or skin inflammation		3
Severe/large scale lesions, severe skin inflammation		HEP
Respiratory tract		
normal		0
Tachypnoea (slight)		1
Tachypnoea (moderate)		2
Dyspnoea (marked) <24h		3
Dyspnoea (marked) >24h		HEP
mild serous ocular or nasal discharge		1
moderate serous to purulent ocular or nasal discharge		2
severe serous to purulent ocular or nasal discharge		3
Environment		
normal		0
Loose stools or diarrhoea		1
haemorrhagic diarrhoea		HEP
Social Behaviour/General condition/Locomotion		
normal		0
Lack of grooming, mildly inactive (tired)		1
Not restricted in mobility, beginning kyphosis (hunched back), mildly inactive and depressed; Reduced interaction with other animals/apathic		2
hunched up back (kyphosis), lethargic, isolation or hyperactive, vocalization on moving		3
severe kyphosis		HEP
Immobility/moribund		HEP
Neurological scoring		
normal		0
mild neurological signs (e.g. head tilt, beginning ataxia, paralysis)		3
moderate to severe neurological signs, ataxic, paralysis, shaking, convulsions, moving in circles		HEP
Other		

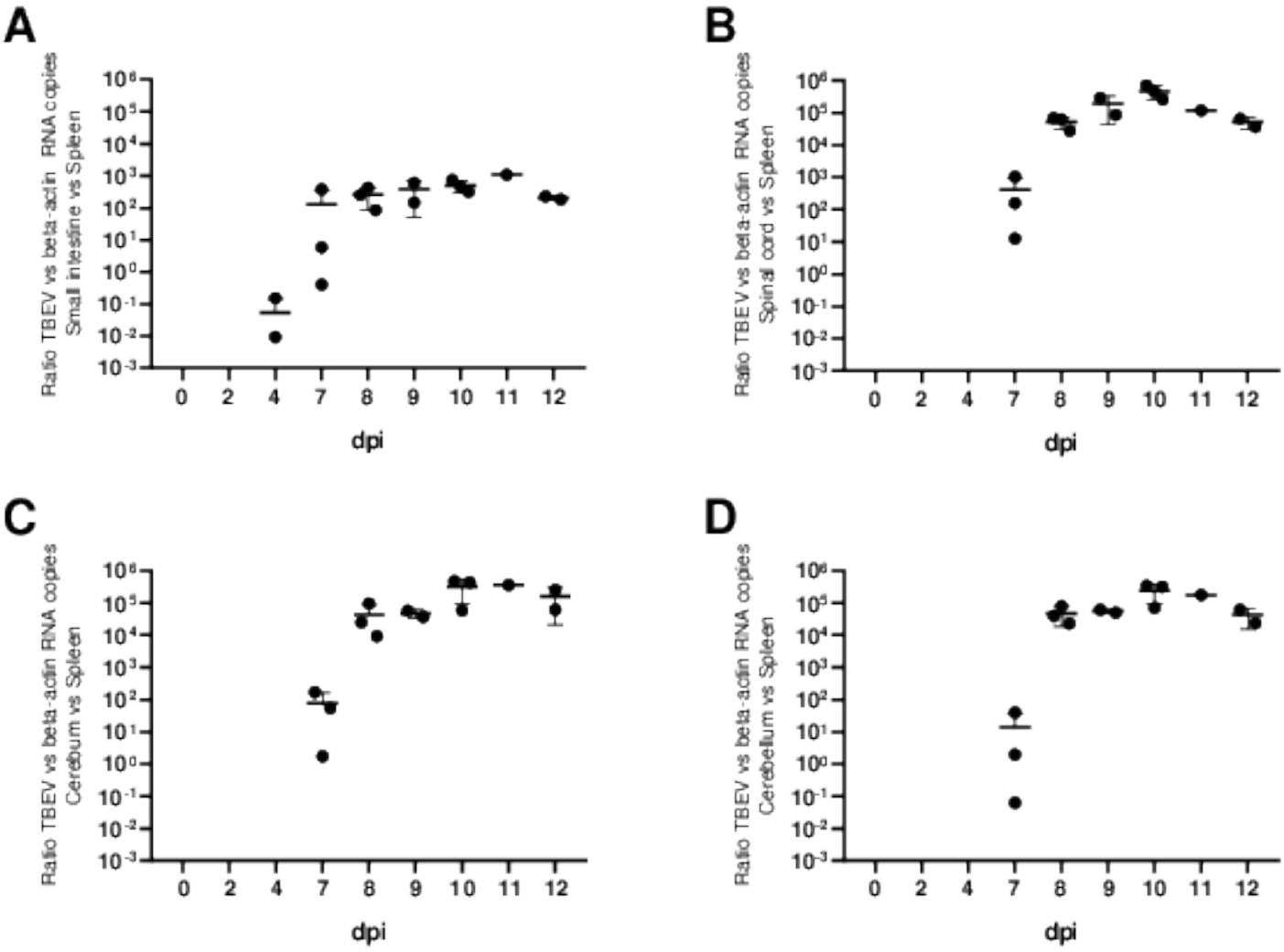
Actions	
CS 1	Review frequency of monitoring
CS 2	≤ 12 h frequency of monitoring
CS 4	consult veterinarian
CS 6 >48 h	Implement humane endpoint
CS=3 in >2 categories (highlighted in grey)	Implement humane endpoint
HEP	Implement humane endpoint

*Im Folgenden werden die Abbruchkriterien aufgrund des Gewichtes im Detail erläutert:

1. „acute weight loss" > 20% in comparison to initial weight" = akuter Gewichtsverlust **>20% innerhalb 24 h** im Vergleich zum initialen Gewicht vor dem Versuch.

2. „weight loss > 25% in comparison to initial weight" = Gewichtsverlust **>25% im Vergleich zum initialen Gewicht vor dem Versuch (sofern Kriterium 1 „acute weight loss >20%" innerhalb 24 h nicht zutrifft**).

   Das Allgemeinbefinden der Tiere sollte ansonsten ohne besonderen Befund sein (d.h. Clinical score in weiteren Kategorien = 0). Sollte das Tier einen Gewichtsverlust zwischen >20% und 25% haben und in anderen Kategorien einen Clinical Score von ≥1, wird das Tier unverzüglich euthanasiert


A
Ratio TBEV vs beta-actin RNA copies
Small intestine vs Spleen
B
Ratio TBEV vs beta-actin RNA copies
Spinal cord vs Spleen
C
Ratio TBEV vs beta-actin RNA copies
Cerebrum vs Spleen
D
Ratio TBEV vs beta-actin RNA copies
Cerebellum vs Spleen
dpi
0 2 4 7 8 9 10 11 12