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Evaluation of "SNAP® Lepto"-ELISA and comparison with MAT and PCR results for diagnosis of leptospiral uveitis in horses using intraocular samples

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Summary: Chronic intraocular leptospiral infection is the most common cause for recurrent uveitis in equids. History and a combination of typical ophthalmological findings can lead to suspected diagnosis of leptospiral induced uveitis. Testing intraocular fluids had been shown to be the most valuable tool for providing evidence of an intraocular leptospiral infection. The most appropriate treatment for recurrences of leptospiral uveitis is vitrectomy, whereas vitrectomy in non-leptospiral uveitis cases is less successful in regard of avoiding further damage to effected eyes. However, in cases in which clinical and ophthalmological findings are not typical for leptospiral induced uveitis, preoperative testing of aqueous fluid can be indicated to evaluate an indication for vitrectomy. Until now, for save sampling of aqueous humour, a general anaesthesia was necessary for paracentesis of the anterior chamber, and a second was necessary for vitrectomy in case of leptospira-positive laboratory results (either antibodies using microscopic agglutination tests (MAT), enzyme-linked immunosorbent assays (ELISA) or polymerase chain reactions (PCR) detecting LipL32). A quick ELISA test, the SNAP® Lepto, has recently been made available to detect antibodies directed against LipL32 of pathogenic leptospires in any species. In order to evaluate the SNAP® Lepto and to test whether it is effective with intraocular samples (aqueous and vitreous humour) from horses, 118 samples from equine eyes suffering from recurrent uveitis and 120 samples from equine control eyes were taken and tested with SNAP® Lepto (detecting antibodies directed against LipL32), MAT (detecting antibodies directed against ten different serovars) and PCR (detecting the LipL32 antigen). If one of these tests was positive, an intraocular leptospiral infection was assumed.The result was that SNAP® Lepto was positive in 114 out of 118 intraocular samples (97 %) from horses suffering from recurrent uveitis. SNAP® Lepto was even superior to MAT, with a sensitivity of 0.97 (MAT 0.93). PCR was positive with fluids from eyes suffering from uveitis in 70% of samples. There were no positive PCR results in any SNAP® Lepto-negative samples from uveitic eyes and the MAT was positive in four SNAP® Lepto-negative samples. On the other hand, SNAP® Lepto gave a positive result in eight MAT-negative samples from eyes with uveitis. Furthermore, none of the antibody tests was positive regarding any sample from the control eyes, and PCR was positive in one single sample from a control eye. It can be concluded that SNAP® Lepto, which gives a result within 10 minutes, is a valuable test method for detecting antibodies directed against LipL32 in intraocular fluids from horses. In the case of a negative result, MAT and PCR can be performed subsequently to reduce false negative results.

Keywords: chronic intraocular leptospiral infection, equine recurrent uveitis (ERU), MAT, ELISA, serum, SNAP® Lepto, anterior chamber fluid, vitreous

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Introduction

Clinical symptoms of acute leptospirosis are rare in horses (*Roberts* et al. 1952, *Williams* 1968, *Brem* et al. 1992, *Bernard* 1993, *Erol* et al. 2015, *Hamond* et al. 2014). However, equine recurrent uveitis (ERU) as a late sequela is well known (*Rimpau* 1947, *Wood* and *Davis* 1950, *Heusser* 1952, *Kalisch* 1952, *Kemenes* and *Tamas* 1952, *Witmer* et al. 1953, *Schebitz* 1954, *Bryans* 1955, *Bolte* 1966, *Williams* 1968, *Morter* et al. 1969, *Williams* et al. 1971, *Halliwell* et al. 1985, *Sillerud* et al. 1987, *Spiess* 2010). Some years ago, leptospiral uveitis has been described as an important uveitic entity of humans and equines (*Verma* and *Stevenson* 2012).

Trans-pars-plana vitrectomy is the most effective treatment option in horses suffering from recurrent uveitis (ERU) to prevent recurrences (*Werry* and *Gerhards* 1991, 1992, *Winterberg* and *Gerhards* 1997, *Frühauf* et al. 1998, *Gerhards* et al. 1999, *Gerhards* and *Wollanke* 2005, *Von Borstel* et al. 2005, *Tömördy* et al. 2010, *Schinagl* 2017). If vitrectomy is performed before irreversible damage of the eyes occurs (especially posterior synechia, the beginning of cataract formation or the beginning of retinal detachment), prognosis for long-term preservation of vision is excellent (*Schinagl* 2017).

It is important when there is a leptospiral-induced uveitis that the leptospiral infection is removed from the vitreous by surgery to achieve permanent absence of uveitis recurrences after vitrectomy. In cases where uveitis is not caused by leptospires (e.g. phacogenic uveitis, most eyes suffering from glaucoma or leopard coat pattern uveitis), vitrectomy cannot or can only slightly influence the course of the disease (*Gerhards* and *Wollanke* 2005, *Gerhards* and *Wollanke* 2006, *Tömördy* et al. 2010, *Baumgart* and *Gerhards* 2014, *Schinagl* 2017). Only in the case of major vitreous opacities ("floaters") vitrectomy might be indicated for removal of these opacities and, thus, improve vision, at least for some time, and delay cataract formation which is caused by the attachment of inflammatory precipitates to the posterior lens capsule (*Werry* and *Gerhards* 1992, *Winterberg* and *Gerhards* 1997, *Schinagl* 2017).

Detection of antibodies directed against leptospires (*Gsell* et al. 1946, *Wood* and *Davis* 1950) and leptospiral DNA in intraocular fluids is not new, but varies considerably between different investigations (*Wollanke* et al. 1998b, *Faber* et al. 2000, *Wollanke* et al. 2001b, *Wollanke* 2002, *Wollanke* et al. 2004, Gesell et al. 2005, 2006, Gilger et al. 2008, Loibl 2009, *Von Borstel* et al. 2010, *Verma* et al. 2012, *Wiehen* 2012, *Polle* et al. 2014, *Roth* et al. 2014, *Baake* et al. 2016, *Dorrego-Keiter* et al. 2016, 2017, *Malalana* et al. 2017). Reasons for this might be the time of sampling (e.g. in the beginning of the disease, while there are ongoing uveitis recurrences or, finally, from blind and shrunken and eyes which are no longer painful), dilution of some the intraocular fluid samples, different tests performed in different laboratories and not using each available test in some investigations, different MAT titre values considered as "positive", different serovars or different leptospira strains used in different laboratories, inconsistent assessment of ocular findings leading to the diagnosis of ERU, and geographical differences.

The medical history and clinical and ophthalmological examinations (*Yager* et al. 1950, *Severin* 1986, *Miller* and *Whitley* 1987, *Lavach* 1990, *Davidson* 1991, 1992, *Spiess* 1997, *Gerhards* and *Wollanke* 2005, *Curling* 2011, *McMullen* and *Fischer* 2017) are usually decisive for suspecting leptospira-induced uveitis (*Wollanke* et al. 2004). In these cases, vitrectomy is indicated without performing any laboratory tests. In a few cases, vitrectomy is indicated just because of vitreous opacities, regardless of the presence of an intraocular leptospiral infection. However, indication for vitrectomy is questionable in cases where there are no typical clinical and ophthalmological findings and lack of medical history, or in horses with a leopard coat pattern, and in most cases of glaucoma.

In these horses, preoperative sampling of aqueous fluid and testing for antibodies directed against leptospires and leptospiral DNA might be indicated to evaluate the prognosis for the absence of uveitis recurrences after surgery. Testing of serum samples by MAT is too unspecific and, thus, inappropriate for the diagnosis of a leptospiral-induced uveitis, because too many clinically sound horses have detectable serum-antibodies using MAT (*Matthews* et al. 1987, *Wollanke* et al. 1998a, 1998b, *Wollanke* et al. 2000, *Wollanke* 2002, *Wollanke* et al. 2004, *Gilger* et al. 2008, *Loibl* 2009, *Malalana* et al. 2017). Furthermore, MAT with serum was negative in about 10% of horses from which leptospires could be cultured with vitreous material (*Wollanke* 2002, *Wollanke* et al. 2004).

It could be shown in earlier investigations that ELISA testing was more sensitive compared to MAT in detecting intraocular anti-leptospiral antibodies (*Loibl* 2009, *Loibl* et al. 2018). A laboratory ELISA quick test (SNAP® Lepto, Fa. IDEXX) for dogs has recently become available (*Winzelberg* et al. 2015, *Curtis* et al. 2015). This ELISA is neither serovar- nor species-specific, but any antibodies directed against the LipL32 antigen of pathogen leptospires and possessing more than one binding site can be detected. The more binding sites the antibodies are equipped with, the better this test should work (e.g. IgM with ten docking sites > IgA with four docking sites as dimer or with two docking sites as monomer > IgG with two docking sites) (IDEXX 2016).

The aim of this study was to evaluate whether this quick ELISA test is appropriate for testing intraocular samples from horses and whether this test could replace the more differentiated but costlier and more time-consuming ELISA testing which offers differentiation between serovars and immunoglobulin classes. Furthermore, as for this discussion understanding and interpretation of the occurrence of intraocular antibodies is essential, a brief discussion about the significance of intraocular antibodies and the Goldmann-Witmer Coefficient is added.

Methods

A total of 118 intraocular samples from 108 equine eyes suffering from leptospiral uveitis were taken. These samples included 107 vitreous samples which could be taken undiluted at the beginning of vitrectomies and 11 aqueous fluid samples which were taken from horses with questionable findings regarding typical symptoms and findings of leptospiral uveitis prior to surgery (in order to check the indication for vitrectomy). In case of positive results, looking either for leptospiral antibodies or leptospiral DNA, no additional vitreous sample was tested during the following vitrectomy for economic reasons. The requirement for classification as "leptospira-induced uveitis" in addition to medical history and clinical and ophthalmological findings indicating a leptospiral uveitis (*Gerhards* and *Wollanke* 2001, 2006) was that there were antibodies directed against leptospires and/or a positive DNA result (PCR for detection of LipL32 which is specific for pathogenic leptospires) in intraocular fluids.

Controls were 120 samples from clinically and ophthalmologically sound eyes of 60 horses. These samples consisted of 20 aqueous fluid samples and 100 vitreous samples. All intraocular samples were tested immediately using the SNAP® Lepto for antibodies directed against the LipL32 of pathogenic leptospires. This test gives a result within 10 minutes, but cannot differentiate between serovars and immunoglobulin classes of the antibodies. The LipL32 is deposited both on the sample spot (Fig. 1) and within the horseradish peroxidase conjugate (Fig. 2). Thus, antibodies in the sample will bind to LipL32 in the enzyme-conjugated fluid and when flowing over the sample point on the sample point as well (Fig. 4 a). Reverse chromatographic flow of lavage fluid and substrate agents takes place after the end of the test unit is pressed down and this induces the colour reaction (Fig. 3, 4, and 5). The control point consists of antibodies to horseradish peroxidase and must turn blue to validate the test. The sample point will turn blue to different degrees if antibodies directed against LipL32 have been in the sample (Fig. 5). Therefore, any antibody directed against LipL32 which has more than one binding site can be detected with the SNAP® Lepto, but the test is neither serovar- nor species-specific.

In this study, solely "positive" and "negative" tests results were used for the evaluation. The different degrees of blue reaction on the sample point (Fig. 5) were not taken into account.

When SNAP® Lepto was completed, identical samples were sent to a DAkkS accredited laboratory (DIN EN ISO/IEC 17025) and tested both for LipL32 using real-time PCR and for antibodies directed against the serovars Grippotyphosa,

Pomona, Australis, Bratislava, Canicola, Copenhageni, Icterohaemorrhagiae, Hardjo, Saxkoebing and Tarassovi using MAT. Antibody titres were considered as "positive" when the MAT result was 1:100 or higher. Any MAT results lower than 1:100 were considered as "negative".

Results of antibody tests and PCR were compared and sensitivity, specificity, and positive and negative predictive values for the presence of a leptospiral-induced uveitis were determined. Leptospiral uveitis was assumed when either one antibody test and/or PCR gave positive results.

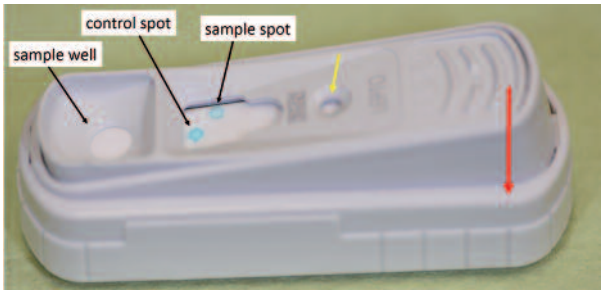

Fig. 1 New test kit with sample well, control spot, sample spot, control hole (yellow arrow) for visualisation of the fluid reaching this point and the site on the test kit which is pressed down (red arrow).
Neuer Testkit mit der Vertiefung, in die das Gemisch aus Probe und Konjugat getropft wird, Kontrollpunkt, Probenpunkt, Kontrollöffnung (gelber Pfeil) um zu sehen, wann die Flüssigkeit hier ankommt und die Seite des Tests, die dann herunter gedrückt werden muss (roter Pfeil).

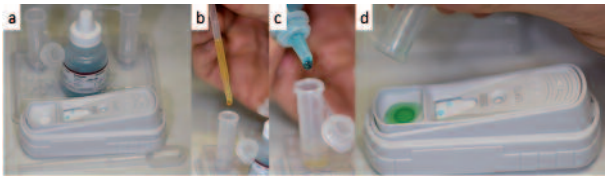

Fig. 2 Complete test kit with blue conjugate fluid and pipette (a). Sample (b) and horseradish perodidase (HRP) conjugate (c) are mixed and placed into the sample well (d).
Kompletter Testkit mit blauem Konjugat und Pipette (a). Die zu untersuchende Probe (b) und Meerettichperoxidase-Konjugat (c) werden vermischt und in die dafür vorgesehene Vertiefung getropft (d).

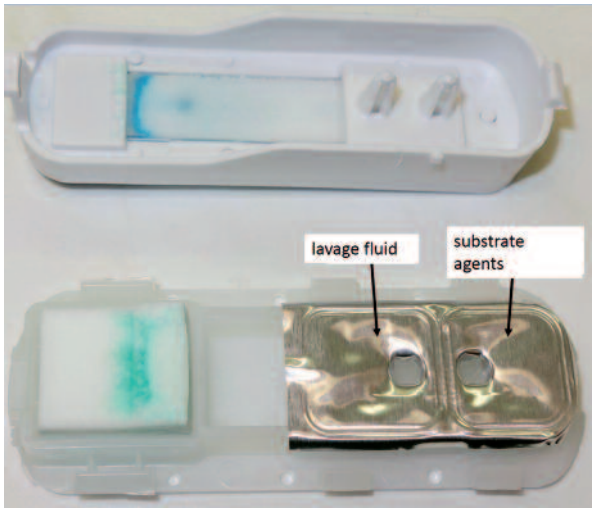

Fig. 3 Opened test kit showing how the flow matrix will be exposed to wash solution and substrate agents after the end of the test kit is pressed down.
Geöffneter Test bei dem man erkennen kann, warum nach herunterdrücken der einen Seite der Testeinheit Waschlösung und Substrat in entgegengesetzter Richtung über die Matrix.

Results

Leptospira-induced uveitis

MAT and SNAP® Lepto ELISA gave about the same results regarding the presence of antibodies directed against leptospires. Only a few samples were either solely positive using MAT (4/118 intraocular samples) or solely positive using SNAP® Lepto (8/118 intraocular samples). Both antibody tests were congruently positive in 106 out of 118 (90%) intraocular samples (Table 1 and 2). At least one of these antibody tests was positive in each sample (100%).

The PCR and antibody tests were congruently positive in 83 out of 118 intraocular samples (70%). The PCR gave a negative result in 35 out of 118 (30%) antibody-positive samples

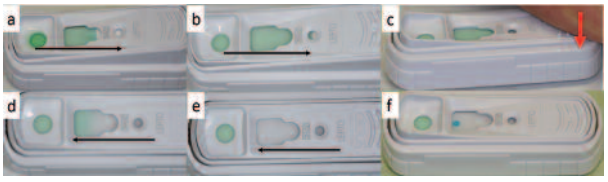

Fig. 4 (a) Three drops of the sample mixed with four drops of the conjugate (LipL32-HRP) which has been placed into the sample well (see Fig.1) are on the flow matrix. (b) When the fluid reaches the control hole (see Fig. 1), the end of the sample kit is pressed down (c), allowing the lavage fluid and substrate agents (see Fig. 3) to flow in the adverse direction (d, e). The control spot (see Fig. 1) must turn blue for the test to be validated. The sample point (see Fig. 1 and 5) turns blue in the presence of antibodies in the sample fluid directed against LipL32.
(a) Drei Tropfen aus der Probe wurden mit vier Tropfen des Konjugats (Meerettichperoxidase-LipL32-Komplexe) gemischt in die dafür vorgesehene Vertiefung gegeben (s. Abb. 1) und fließen nun über die Matrix. (b) Wenn die Flüssigkeit die Kontrollöffnung erreicht (s. Abb. 1) wird das Ende des Testkits heruntergedrückt (c), was den retrograden Fluss der Waschlösung und des Substrates initiiert (d, e). Der Kontrollpunkt (s. Abb. 1) muss blau werden, sonst hat der Test nicht funktioniert. Der Probenpunkt (s. Abb. 1 und 5) färbt sich blau, wenn Antikörper in der Probenflüssigkeit vorhanden waren.

Fig. 5 Results using SNAP® Lepto: 1 negative, 2 slightly positive, 3 – 6 different degrees but clearly positive.
Ergebnisse bei Verwendung des SNAP® Lepto: 1 negativ, 2 geringgradig positiv, 3 – 6 eindeutig positive Ergebnisse mit unterschiedlichen Graden.

and no sample was solely PCR-positive. Each PCR-positive sample had given a positive SNAP® Lepto result previously. Looking at the negative MAT results, only two intraocular samples were PCR-positive. Sensitivity and specificity were very high for both antibody tests and a little bit lower for PCR (see Table 1). The SNAP® Lepto gave the highest sensitivity (0.97).

Controls

Antibodies directed against leptospires could not be detected with either MAT or SNAP® Lepto in any sample from control eyes (0/120), but PCR gave a positive result with one single sample from a control eye (Tables 1 and 2).

Discussion

Proper case selection, which means carrying out vitrectomy to prevent further uveitis attacks in cases of leptospiral uveitis, is the key to successful trans-pars-plana vitrectomy in horses (*Frühauf* et al. 1998, *Wollanke* et al. 2004, *Gerhards* and *Wollanke* 2005, *Tömördy* et al. 2010, *Schinagl* 2017).

The results in this study prove the relatively cheap SNAP® Lepto to be useful and convenient for detecting antibodies directed against pathogenic leptopires in intraocular fluids. This test can be used for clinical purposes instead of the more complex and expensive ELISA testing (*Kettner* 1997, *Loibl* 2009) which, however, can differentiate between serovars and immunoglobulins. SNAP® Lepto shows high sensitivity

Table 1 Results of testing intraocular samples from horses with leptospiral uveitis and from clinically and ophthalmoscopically sound controls using MAT, SNAP® Lepto and PCR, considering sensitivity and specificity as well as positive and negative predictive values. (V = vitreous sample, AF = aqueous fluid, i. o. samples = vitreous and aqueous fluid samples together, "+" = positive result, "-" = negative result)
Ergebnisse der MAR-, SNAP® Lepto- und PCR-Untersuchungen von intraokularen Proben aus klinisch und ophthalmoskopisch gesunden Kontrollaugen und an leptospiren-bedingter Uveitis erkrankten Augen unter Berücksichtigung der Sensitivität und Spezifität sowie des positiven und negativen prädiktiven Werts. (V = Glaskörper, AF = Kammerwasser, i. o. samples = Glaskörper- und Kammerwasserproben zusammen, „+" = positives Testergebnis, „-" = negatives Testergebnis)

	Test	Sound control eyes			Leptosiral uveitis			Sensitivity	Specificity	Positive predictive value	Negative predictive value
		[n] in total	[n] +	[n] -	[n] in total	[n] +	[n] -				
V (n = 207)	MAT	100	0	100	107	99	8	0.93	1.00	1.00	0.93
	SNAP	100	0	100	107	104	3	0.97	1.00	1.00	0.97
	PCR	100	1	99	107	81	26	0.76	0.99	0.99	0.79
AF (n = 31)	MAT	20	0	20	11	11	0	1.00	1.00	1.00	1
	SNAP	20	0	20	11	10	1	0.91	1.00	1.00	0.95
	PCR	20	0	20	11	2	9	0.22	1.00	1.00	0.69
i. o. samples (n = 238)	MAT	120	0	120	118	110	8	0.93	1.00	1.00	0.94
	SNAP	120	0	120	118	114	4	0.97	1.00	1.00	0.96
	PCR	120	1	119	118	83	35	0.70	0.99	0.99	0.77

Table 2 Comparison of the test results of MAT, SNAP® Lepto and PCR examinations using intraocular samples from horses with leptospiral uveitis and from clinically and ophthalmoscopically sound controls. (V = vitreous samples, AF = aqueous fluid samples, i. o. samples = vitreous and aqueous fluid samples together, l. u. = leptospiral uveitis, "+" = positive results, "-" = negative results)Vergleich der Ergebnisse der MAR-, *SNAP® Lepto- und PCR-Untersuchungen von intraokularen Proben von Pferden mit leptospirenbedingter Uveitis und klinisch und ophthalmoskopisch augengesunden Kontrollen. (V = Glaskörper, AF = Kammerwasser, i. o. samples = Glaskörper- und Kammerwasserproben zusammen, l. u. = leptospirenbedingte Uveitis, „+" = positives Testergebnis, „-" = negatives Testergebnis)*

	MAT- & ELISA+	MAT+ & ELISA-	MAT+ & ELISA+	MAT- & ELISA-	MAT- & PCR+	ELISA- & PCR+	MAT+	ELISA+	PCR+
V l. u. (n = 107)	8	3	96	0	2	0	3	8	0
V controls (n = 100)	0	0	0	100	1	1	0	0	1
AF l. u. (n = 11)	0	1	10	0	0	0	1	0	0
AF controls (n = 20)	0	0	0	20	0	0	0	0	0
i. o. l. u. (n = 118)	8	4	106	0	2	0	4	8	0
i. o. controls (n = 120)	0	0	0	120	1	1	0	0	1

and specificity as well as positive and negative predictive values and is, thus, a very reliable test. SNAP® Lepto may be inappropriate for some scientific purposes, and the information gain is lower, however, serovar differentiation and differentiation of immunoglobulins within detectable antibodies is not important for clinical differential diagnostics (leptospiral or non leptospiral uveitis).

Comparing SNAP® Lepto and PCR, there was only one single sample (belonging to a control eye) in which there was LipL32 itself detectable, but no antibodies against this antigen. Therefore, it can be concluded that PCR does not give much more information than SNAP® Lepto. Comparing MAT and SNAP® Lepto results, there was a solely positive MAT result in 4 out of 118 samples (3,4%). Therefore, when a negative SNAP® Lepto result is obtained, it is reasonable to perform a MAT afterwards to reduce false-negative results. Positive PCR results might also occur in the case of a negative antibody result in rare cases. It is possible that positive PCR results without accompanying antibodies occur especially in the early stages of intraocular infections and before ERU appears because immune reactions and antibody production have not already developed to a detectable amount.

One big advantage of the SNAP® Lepto is that it can be performed within 10 minutes, meaning that this can be performed immediately prior to surgery in the case of a preoperatively indicated aqueous fluid examination. If SNAP® Lepto is positive, sending for MAT is not necessary. Thus, in the case of a positive SNAP® Lepto result, surgery can be performed immediately after a positive test result, and a second general anaesthesia is avoidable. This reduces the perioperative risk significantly as recovery from anaesthesia can be dangerous to horses. Furthermore, laboratory costs and time of hospitalisation are minimised.

In the case of a negative test result using SNAP® Lepto, this might be false-negative and additional laboratory testing using MAT, PCR and the more differentiating ELISA tests are helpful in reducing false-negative results. In these cases, a second general anaesthesia is unavoidable if a positive test result comes from the laboratory some days later. However, in most cases, the diagnosis of a leptospira-induced uveitis will be possible within 10 minutes using SNAP® Lepto and testing aqueous fluid.

For some inexplicable reason, recently the calculation of the Goldmann-Witmer coefficient had been revisited as a precondition for the diagnosis of a leptospira-induced uveitis (*Gilger* 2018, *Malalana* 2018) as it had been advocated in human ophthalmology for diagnosis of infectious uveitis (*Rothova* et al. 2008). However, an intraocular antibody production in equids had been proved in earlier studies (*Wollanke* 2002, *Wollanke* et al. 2004) and in addition, PCR and culture results are positive in high percentages (*Brem* et al. 1999, *Wollanke* et al. 1999, *Wollanke* et al. 2000, *Wollanke* and *Gerhards* 2001a, 2001b, *Wollanke* 2002, *Hartskeerl* et al. 2004, *Wollanke* et al. 2004, *Roczek* 2008, *Von Borstel* et al. 2010, *Popp* et al. 2013, *Baake* et al. 2016) and there is ultrastructural evidence of leptospires inside the vitreous of ERU eyes (*Niedermaier* et al. 2006, *Brandes* et al. 2007).

Furthermore, no leptospiral antibodies could be detected in intraocular samples from eyes with different ocular diseases causing a breakdown of the blood-ocular barrier (e.g. eyes suffering from glaucoma, eyes with severe intraocular bleeding a few days after the haemorrhage, other uveitis form such as phacogenic uveitis, severe uveitis accompanying rhodococcus or micronema deletrix infections), while there had been positive MAT results with serum from the same horses (*Wollanke* et al. 1998a, 1998b, *Wollanke* 2002, *Wollanke* et al. 2004).

Additionally, there are antibodies solely directed against leptospires in intraocular fluids in horses with leptospira-induced uveitis, but no antibodies directed against other infectious agents which were present in the corresponding serum samples at the same time. If antibodies were present because of a damaged blood-ocular barrier and, thus, leakage from the blood into the eye, there must have been different antibodies in the intraocular fluids which had been in serum samples. It could be shown that antibodies directed against Toxoplasma, Herpesvirus and Bornavirus were detectable in the serum, but never intraocularly in the corresponding samples from the same horses, while solely MAT results were positive with the intraocular samples (*Wollanke* et al. 2000).

A dysfunctional blood-ocular barrier cannot be the reason for the much higher MAT results with intraocular fluids compared to MAT results with corresponding serum samples from the same horses (*Gsell* et al. 1946, *Heusser* 1948, *Witmer* 1955, *Davidson* et al. 1987, *Wollanke* et al. 1998b). There are often even different serovars detectable in serum and corresponding samples from intraocular fluids (*Wollanke* et al. 1998b, *Wollanke* 2002, *Wollanke* et al. 2004). This also cannot be explained by a breakdown of the blood-ocular barrier.

Finally, there is hardly any albumin in the intraocular fluids and total protein in the vitreous is much lower than in the serum in the early stages of the disease. Nevertheless, in these eyes, high MAT titres are detectable. A damaged blood-ocular barrier would lead to leakage of albumin prior to the larger globulins, for example, agglutinating antibodies (*Wollanke* et al. 1998a, 1998b, *Wollanke* 2002, *Wollanke* et al. 2004). As this is not the case, an intraocular antibody production against leptospires is obvious.

The clinical signs of ERU are clear in eyes with considerable vitreous cloudiness and, thus, possibly impaired function of the blood-ocular barrier, and any preoperative laboratory testing would be superfluous.

Consequently, calculation of the Goldmann-Witmer coefficient is not necessary for diagnosing a leptospira-induced uveitis but testing of intraocular fluids is decisive. Additional serum testing just increases costs for laboratory tests and does not lead to more certainty whether there is an intraocular leptospiral infection or not.

Conclusion

SNAP® Lepto gives very similar or even more sensitive and specific results compared to the MAT regarding antibody detection using intraocular fluids. As the SNAP® Lepto result is achieved within 10 minutes, it is possible to test aqueous

humour for antibodies directed against LipL32 and – in the case of a positive test result – to perform vitrectomy within one single general anaesthesia. In doubtful cases, however, using SNAP® Lepto, unnecessary vitrectomies can be avoided.

Conflict of interest:

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper. IDEXX neither initiated nor sponsored this study.

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