Evidence-based early clinical detection of emerging diseases in food animals and zoonoses

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SYNOPSIS

In case diseases of food-producing animals or zoonoses (re-)emerge, early clinical decision making is of major importance. In this particular condition, it is difficult to apply a classical evidence-based veterinary medicine process, because of a lack of available published data. A method based on the partition of field clinical observations (evidences) could be developed as an interesting alternative approach. The classification and regression tree (CART) analysis was used to improve the early clinical detection of two selected emerging diseases: bovine spongiform encephalopathy (mad cow disease) and bluetongue due to the serotype 8-virus in cattle.

ABSTRACT

Background: In case diseases of food-producing animals or zoonoses (re-)emerge, early clinical decision making is of major importance. In this particular condition, it is difficult to apply a classical evidence-based veterinary medicine process, because of a lack of available published data. **Objective**: A method based on the partition of field clinical observations (evidences) could be developed as an interesting alternative approach. **Method and principal findings:** The classification and regression tree (CART) analysis was used to improve the early clinical detection of two selected emerging diseases: bovine spongiform encephalopathy (mad cow disease) and bluetongue due to the serotype 8-virus in cattle. **Conclusion and significance:** The use of CART analysis is a way to improve the early clinical detection of diseases of food-producing animals or zoonoses as well as conditions of emergence. The development of a veterinary structured, informed and interactive clinical platform is highly suggested.

INTRODUCTION

Evidence-based veterinary medicine (EBVM) is the application of evidence-based medicine (EBM) to the veterinary field (1). By definition, it is the conscientious, explicit and judicious use of the best scientific evidence to inform clinical decisions with a view to improve the clinical outcome at the individual level (2-3). However, in the veterinary profession, a great deal of time is spent in making diagnostic, therapeutic and preventive decisions in a complex and uncertain environment where optimal evidence often lacks (4).

Medical care is the art of making decisions without adequate information (5). Medical decision making has been studied extensively and follows a mainstream trend, labelled 'rational optimising' (6). It is usually based on cognitive rational models, such as decision analysis, decision tables, decision trees and Bayes' theorem (7-11). When decision refers to diagnosis, the consideration of the possible causes of a disease, its prevalence and an initial evaluation of clinical signs will lead to a differential diagnosis about which clinical judgment, informed by evidence clinical data, is exercised (3). Diagnosis may involve the choice and interpretation of an appropriate confirmatory diagnostic test.

To detect and identify emerging or rare diseases, a good clinical approach is essential as few biological and epidemiological data and\or laboratory tests are available. The approach aims at establishing the limits between normality and abnormality as veterinarians cannot relate the clinical signs to those of a known disease or to their experience. These limits should be built on the ability to detect biological variations in physiological and environmental conditions. The various actors involved in epidemiosurveillance networks (e.g. breeders, veterinarians, and slaughterhouse staff) should be prepared to this clinical approach to fulfil their responsibility in health monitoring (12). Part of this training should develop knowledge of disease biology and epidemiology, and skills in a rigorous, standardized and evidence-based clinical approach including that of differential diagnosis (13-16).

However, since with emerging diseases, the implementation of classical EBVM is difficult because few published cases are available and/or accessible via web searches, other options are necessary.

The current paper aims to describe a method to improve the early clinical detection of emerging diseases in food animals and zoonoses. This approach is based on the analysis of field clinical observations collected on the first cases suspected of disease using a method called “classification and regression tree” (CART) (17-19). Those clinical facts become the only evidences available. Two practical examples are developed to illustrate the feasibility of the method in cattle. Future prospect is also proposed like the implementation of a structured, well-informed and interactive veterinary web clinical data mining platform.

CASE DESCRIPTION

Two examples are developed to illustrate the use of CART analysis for stimulating the early warning of emerging animal diseases. This is a key parameter of health control strategy (20). CART analysis is a non-linear and non-parametric model fitted by binary recursive partitioning of data (including clinical signs). Using CART 6.0 software (Salford Systems, San Diego, CA, USA), the analysis successively splits the dataset into increasingly homogeneous subsets until it is stratified and meets specified criteria (clinical signs) (**Figure 1**). Further details about CART are presented in previously original papers or reviews (17-19, 21).

Case 1: Early detection of bovine spongiform encephalopathy

Background: Bovine spongiform encephalopathy (BSE) emerged in 1986 (22). It is a neurodegenerative disease characterised by a very long incubation period compared to the life of the host species (23). BSE started a dramatic chain of events in the United Kingdom and subsequently in other countries (24). The peak of interest was the discovery of its potential

zoonotic character after the first description of a new variant of Creutzfeldt-Jakob disease (vCJD) in 1996 (25-27). The presence of clinical signs seems to be linked to the localisation and degree of vacuolisation of neurones. The main warning signs are psychic disorders (apprehension, temperament change, abnormal ear position and abnormal behaviour), sensory disorders (exaggerated responses to stimuli, excessive licking) as well as postural and locomotion abnormalities (ataxia and tremors). Their identification requires a clinical approach: a thorough veterinary clinical examination of the animal when on a halter and when moving in an uncustomary environment (16).

Now the evolution of BSE incidence in many European countries is in decline (28). Because of the favourable BSE epidemiological situation of most Member States in the European Union, a lowering of control measures, by reducing testing procedure, was recently suggested. However, in such a context, the reporting of clinically suspected cattle by the veterinarians is the most common method for detecting sporadic cases of BSE (18). The improvement of clinical diagnosis and decision-making remains crucial.

Veterinary data collection: A comparison of clinical patterns captured by veterinarians, consisting in 25 signs, was carried out between BSE cases confirmed in Belgium before October 2002 (N = 30), and 272 suspected cases that were subsequently determined to be histologically, immunohistochemically, and scrapie-associated-fiber negative (10).

Epidemiological methods and principal findings: Seasonality in reporting suspected cases was observed, with more cases being reported during wintertime when animals were kept indoors. The median duration of illness was 30 days. Using odds ratio, the 10 most relevant signs of BSE were kicking in the milking parlour, hypersensitivity to touch and/or sound, head shyness, panic-stricken response, reluctance to enter in the milking parlour, abnormal ear movement or carriage, increased alertness behaviour, reduced milk yield, teeth grinding and

123 temperament change. Ataxia did not appear to be a specific sign of BSE. A classification and
124 regression tree was constructed by epidemiologists using the following four features: age of
125 the animal, year of birth, number of relevant BSE signs noted, and number of clinical signs
126 typical of listeriosis reported. The model presented a 100% sensitivity and a 85% specificity
127 (**Figure 2**).

128 **Veterinary significance:** The originality of the approach resides in the fact that, first; it
129 involved both veterinarians and epidemiologists. Secondly, it offers an explorative and
130 interactive tool based of clinical observations (evidences) captured by veterinarians and, then,
131 the results and conclusions arrived at are independent of BSE prevalence, through the use of
132 odds ratios. The late feature is especially appealing for rare events. A similar decision tree,
133 allowing the distinction of 'highly suspected BSE cases' from all other suspected BSE cases,
134 could be applied in other countries, with or without the use of rapid tests. The continued
135 addition of standardized clinical data by veterinarians would permit further improvement of
136 the current model tree, even if the clinical BSE pattern would be modified in time. Based on
137 the CART analysis results, veterinarians could more appropriately identify affected cows and
138 retrieve them from the food chain in a public health perspective.

139 ***Case study 2: Early detection of bluetongue***

140 **Background:** Bluetongue (BT) is a non-contagious disease affecting ruminants and is caused
141 by the bluetongue virus (BTV). BTV is transmitted by blood-feeding midges of the genus
142 *Culicoides* (Diptera: *Ceratopogonidae*) (29). A broad spectrum of wild and domestic
143 ruminants can be infected and severe clinical signs are mainly seen in certain breeds of sheep
144 and some *Cervidae* species (30-31). The severity of infection depends on various factors, such
145 as species, breed, age, nutritional and immune status of animals, and environmental stresses,
146 as well as the virulence of the BTV strain involved (32). Although clear differences in
147 virulence of BTV isolates are known, the determinants of virulence are still poorly defined

(32). Clinical manifestations are closely linked to virus-induced vascular injuries and the role of species-specific endothelial cell-derived inflammatory and vasoactive mediators has been highlighted (33). The European BTV-8 outbreak was characterised by peculiar features (34). Among these features, a remarkable severity of the lesions in cattle was noticed (35).

Veterinary data collection: Forty-one cattle from seven Belgian farms and two French farms confirmed as infected with bluetongue virus serotype 8 (BTV-8) were monitored from the onset of clinical signs in order to describe the disease pattern (19). On each visit, a standardised clinical form was filled in for each animal by a veterinarian (**Table I**) (36).

Epidemiological methods and principal findings: A clinical score was calculated for every week until the end of clinical signs. A CART analysis was conducted by epidemiologists to determine the most important clinical signs every week for the first seven weeks. The highest scores were recorded within two weeks of clinical onset. The first recorded clinical signs were quite obviously visible (conjunctivitis, lesions of nasal mucosa and nasal discharge). Skin lesions, a drop in milk production and weight loss appeared later in the course of the disease. A biphasic pattern regarding nasal lesions was noticed: the first peak concerned mainly congestive and ulcerative lesions, whereas the second peak mainly concerned crusty lesions.

Veterinary significance: These results should ensure a more accurate detection of BT in cattle by veterinarians in order to increase the early detection of emerging diseases (**Table II**).

DISCUSSION AND CONCLUSION

The clinical expression of a disease in an animal depends on several parameters: the nature of the causal agent (dose, virulence) (37), the location of induced lesions (38), the host (resistance, general condition, immune status) and the environment; certain clinical signs may be exacerbated when the environment of the animal is altered (39-40). The quality of observation plays an essential role and is proportional to the breeders' and veterinarians' level

of information, awareness and training. The intensity of observation is also important, and seems to depend directly on herd size. According to the United States of America, National Animal Health Monitoring System (NAHMS), the rate of neurological problems in breeding females in beef herds, expressed in affected cattle per thousand, doubles when herd size is less than 100 heads, and is nil when herd size is over 300 heads (41). In addition to these parameters, there is a degree of variability that depends on the individual animal and the observer (clinical picture, pre-patent phase and course of the disease). To improve knowledge regarding diseases, especially (re-)emerging animal diseases, it is important: *i)* to improve awareness, training and information available for breeders and veterinarians, *ii)* to use a uniform method for clinical examination by veterinarians, *iii)* to make more systematic use of confirmatory diagnostic tests, *iv)* to create sentinel networks of highly-motivated breeders and veterinarians, *v)* to transcribe the results of observations in a codified and standardised form, regarding both nature and course, *vi)* to compile and validate existing information by epidemilogists *vii),* to enrich a relational database and *viii),* to discuss actual experience in a focus group.

In case of early clinical detection of emerging animal diseases, an EBVM approach is difficult to perform. However an alternative approach based on new structured and harmonized clinical observations (evidence) should be used (standardized clinical form compiled by veterinarians). With two practical examples we demonstrated the usefulness of joint effort involved veterinarians and epidemiologists in CART analysis to improve the early clinical detection of (re-) emerging animal diseases. The strategy is based on analysis of clinical observations (evidences) captured by veterinarians in the field. Selection criteria are based on signs captured by a structured and harmonized clinical form. A presumptive clinical diagnosis performed by veterinarians implies confirmatory diagnostic test(s). Results are analyzed taking into account all clinical signs registered. The CART analysis carried out by

198 epidemiologists allows producing a robust clinical tree that improves the early clinical
199 detection of diseases by any veterinarian who has not faced the considered emerging disease
200 before.

201 The CART approach is characterised by *i*) its exploratory and interactive aspects, *ii*)
202 its independence from sample size and disease prevalence, which is usually imperfectly
203 known, and *iii*), its spatio-temporal universality (adaptation is possible when the clinical
204 profile of disease evolves in function of time or region; adaptation is also possible for other
205 diseases). The use of tools to improve the detection of (re-)emergent diseases will lead to
206 more effective veterinary epidemiosurveillance networks. The efficacy of these networks
207 requires regular evaluations together with the elaboration and a continuous follow-up of
208 performance indicators. The recent episodes of both human and animal (re-)emergent diseases
209 have also highlighted the important role of global health information systems. These systems
210 require abilities, resources, collaborative and coordinated actions of medical and veterinary
211 regulatory authorities.

212 To improve early clinical detection of (re-)emerging diseases, a future prospect should
213 consist in developing a veterinary structured and informed clinical platform. Whilst some
214 interesting diagnostic support systems for veterinary medicine exist, like the "Consultant"
215 support system from the Cornell College of Veterinary Medicine (http:
216 www.vet.cornell.edu/consultant/consult.asp) (42), no interaction and partition of clinical data
217 are currently available.

218 Facing the emergence of diseases, the translation of the support system to an interactive
219 platform should be interesting. Involving sentinel veterinarians in this platform is crucial.
220 Veterinarians should be stimulated in a pilot research project to ensure the collection of field
221 clinical data through the filling of structured and harmonized clinical forms. The connection
222 between validated clinical data and results of confirmatory diagnostic tests using CART

223 analysis by epidemiologists permits to build useful clinical decision trees to improve the
224 evidence-based early clinical detection of diseases of food-producing animals in the field.
225 More interactions between veterinarians and epidemiologists should be stimulated in a
226 clinical perspective.

227

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230

231 **REFERENCES**

232 1. Cockcroft P., Holmes M. Handbook of Evidence-Based Veterinary Medicine.
233 Blackwell Publishing; Oxford, UK:2003

234 2. Sackett DL, Rosenberg WM, Gray JA, Haynes RB, Richardson WS. Evidence based
235 medicine: what it is and what it isn't. BMJ 1996;312(7023):71-72.

236 3. Petrie A., Watson P. Additional techniques. In Statistics for veterinary and animal
237 science. Volume 14. 2nd edition. Edited by Science B. Edinburgh: Blackwell Science,
238 2006. p.191-211.

239 4. Vandeweerd JM, Kirschvink N, Clegg P, Gustin P, Vandenput S, Saegerman C. Is
240 evidence-based medicine so evident in veterinary research and practice? History,
241 obstacles and perspectives; Vet J, 2011, in press.

242 5. Sox H.C. Decision-making: A comparison of referral practice and primary care.
243 Journal of Family Practice 1996;42:155-160.

244 6. Janis I.L., Mann L. Decision making: A psychological analysis of conflict, choice, and

commitment. New York: The Free Press; New York, USA:1977.

7. Eddy D.M. Clinical decision making: From theory to practice. Anatomy of a decision. JAMA 1990; 263:441-443.

8. Clarke J.R., Rosenman D.L. A scientific approach to surgical reasoning. Resolving tradeoffs - decision trees and decision analysis. Theoretical Surgery 1991;6:110-115.

9. Cantor S.B. Decision analysis: Theory and application to medicine. Prim Care 1995;22:261-270.

10. Saegerman C., Speybroeck N., Roels S., Vanopdenbosch E., Thiry E., Berkvens D. Decision support tools in clinical diagnosis in cows with suspected bovine spongiform encephalopathy. J Clin Microbiol 2004; 42:172-178.

11. Abrahantes J.C., Aerts M., van Everbroeck B., Saegerman C., Berkvens D., Geys H., Mintiens K., Roels S., Cras P. Classification of sporadic Creutzfeld-Jakob disease based on clinical and neuropathological characteristics. Eur J Epidemiol 2007;22:457-465.

12. Commission Européenne. Règlement 999/2001/CE du 22 mai 2001 du Parlement européen et du Conseil fixant les règles pour la prévention, le contrôle et l'éradication de certaines encéphalopathies spongiformes transmissibles. Journal officiel de l'Union européenne 2001;L147:1-40.

13. Stöber M. Symptomatologie différentielle de quelques affections du système nerveux des bovins. Annales de Médecine Vétérinaire 1987;131:401-410.

14. Brown C. Importance des maladies émergentes pour la santé publique et animale et pour les échanges commerciaux. 69ème Session Générale du Comité International de

l'Organisation mondiale de la santé animale, 27 mai au 1er juin 2001, Paris, document 69 SG/9 OIE, 6 pages.

15. Schelcher F., Andreoletti O., Cabanie P., Tabouret G. Démarche diagnostique dans les maladies nerveuses des bovins. *In* Proceedings de la Société Française de Buiâtrie. Paris : 2001, p.229-240.

16. Saegerman C., Claes L., Dewaele A., Desmecht D., Rollin F., Hamoir J., Gustin P., Czaplicki G., Bughin J., Wullepit J., Laureyns J., Roels S., Berkvens D., Vanopdenbosh E., Thiry E. Diagnostic différentiel des troubles à expression nerveuse dans l'espèce bovine en Europe occidentale. Rev sci techn Off Int Epiz 2003;22:61-82.

17. Porter R.S., Leblond A., Lecollinet S., Tritz P., Cantile C., Kutasi O., Zientara S., Pradier S., van Galen G., Speybroek N., Saegerman C. Clinical diagnosis of West Nile Fever in equids by classification and regression tree analysis and comparative study of clinical appearance in three European countries. Transboundary and Emerging Diseases 2011;58:197-205.

18. Saegerman C., Porter S.R., Humblet M.-F. The use of modelling to evaluate and adapt strategies of animal disease control. Rev sc tech Off int Epiz 2011 ;30(2) :555-569.

19. Zanella G., Martinelle L., Guyot H., Mauroy A., De Clercq K., Saegerman C. Clinical pattern characterisation of cattle naturally infected by BTV-8. Vet Microbiol 2011, Submitted.

20. Merianos A. Surveillance and response to disease emergence. Current Topics In Microbiology and Immunology 2007;315:477-508.

289 21. Speybroeck N., Berkvens D., Mfoukou-Ntsakala A., Aerts M., Hens N., Huylenbroeck
290 G.V. & Thys E. Classification trees versus multinomial models in the analysis of
291 urban farming systems in central Africa. Agricultural Systems 2004;80:133-149.

292 22. Wells G.A.H., Scott A.C., Johnson C.T., Gunning R.F., Hancock R.D., Jeffrey M.,
293 Dawson M., Bradley R. A novel progressive spongiform encephalopathy in cattle. Vet
294 Rec 1987;121:419-420.

295 23. Lasmézas C.I. The transmissible spongiform encephalopathies. Rev Sci Techn Off
296 Int Epiz 2003;22(1):23-36.

297 24. Prince M.J., Bailey J.A., Barrowman P.R., Bishop K.J., Campbell G.R., Wood J.M.
298 Bovine spongiform encephalopathy. Rev Sci Techn Off Int Epiz 2003;22(1):37-60.

299 25. Bruce M.E., Will R.G., Ironside J.W., McConnell I., Drummond D., Suttie A.,
300 McCardle L., Chree A., Hope J., Birkett C., Cousens S., Fraser H., Bostock C.J.
301 Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent.
302 Nature 1997;389:498-501.

303 26. Scott M.R., Will R., Ironside J., Nguyen H.-O.B., Tremblay P., Dearmond S.J., Prusiner
304 S. Compelling transgenetic evidence for transmission of bovine spongiform
305 encephalopathy prions to humans. Proc Natl Acad Sci USA 1999;96:15137-15142.

306 27. Hill A.F., Desbrusbais M., Joiner S., Sidle K.C.L., Gowland J., Collinge L., Doey L.J.,
307 Lantos P. The same prion strain causes vCJD and BSE. Nature 1997;389:448-450.

308 28. Saegerman C., Speybroeck N., Vanopdenbosch E., Wilesmith J., Berkvens D. Trends
309 in age-at-detection in Bovine Spongiform Encephalopathy cases: a useful indicator of
310 the epidemic curve. Vet Rec 2006;159:583-587.

311 29. Mellor P.S., Boorman J., Baylis M. Culicoides biting midges: their role as arbovirus
312 vectors. Annu Rev Entomol 2000;45:307–340.

313 30. Howerth E.W., Greene C.E., Prestwood A.K. Experimentally induced bluetongue
314 virus infection in white-tailed deer: coagulation, clinical pathologic, and gross
315 pathologic changes. Am J Vet Res 1988;49:1906–1913.

316 31. Saegerman C., Bolkaerts B., Baricalla C., Raes M., Wiggers L., de Leeuw I.,
317 Vandenbussche F., Zimmer J.-Y., Haubruge E., Cassart D., De Clercq K., Kirschvink
318 N. The impact of naturally-occurring, trans-placental bluetongue virus serotype-8
319 infection on reproductive performance in sheep. Vet J 2011;187:72–80.

320 32. Maclachlan N.J., Drew C.P., Darpel K.E., Worwa G. The pathology and pathogenesis
321 of bluetongue. J Comp Pathol 2009;141:1–16.

322 33. DeMaula C.D., Leutenegger C.M., Bonneau K.R., MacLachlan N.J. The role of
323 endothelial cell-derived inflammatory and vasoactive mediators in the pathogenesis of
324 bluetongue. Virology 2002;296:330–337.

325 34. Dal Pozzo F., Saegerman C., Thiry E. Bovine infection with bluetongue virus with
326 special emphasis on European serotype 8. Vet J 2009;182:142–151.

327 35. Elbers A.R., Backx A., Meroc E., Gerbier G., Staubach C., Hendrickx G., van der
328 Spek A., Mintiens K. Field observations during the bluetongue serotype 8 epidemic in
329 2006. I. Detection of first outbreaks and clinical signs in sheep and cattle in Belgium,
330 France and the Netherlands. Prev Vet Med 2008;87:21–30.

331 36. Saegerman C., Mauroy A., Guyot H. Appendix. Bluetongue in ruminants: a
332 standardised clinical report form for the use in different species. In: *Bluetongue in*
333 *northern Europe*. World Organization for Animal Health and University of Liege

(ed.), Paris, France, 2008, 82-87.

37. Schlech W.F. Listeriosis epidemiology, virulence and the significance of contaminated foodstuffs. J. Hosp.Infect. 1991;19:211-224.

38. George L.W. Localization and differentiation of neurologic diseases. *In* Large animal internal medicine, 2nd Ed. Mosby-Year Book, Saint Louis, 1996:142-170.

39. Kimberlin R.H. Bovine spongiform encephalopathy. *In* Transmissible spongiform encephalopathies of animals. Rev sci tech Off int Epiz 1992;11(2):347-390.

40. Saegerman C., Dechamps P., Vanopdenbosch E., Roels S., Petroff K., Dufey J., Van Caeneghem G., Devreese D., Varewyck H., De Craemere H., Desmedt I., Cormann A., Torck G., Hallet L., Hamerijckx M., Leemans M., Vandersanden A., Peharpre D., Brochier B., Costy F., Muller P., Thiry E. & Pastoret P.-P. Épidémiosurveillance de l'encéphalopathie spongiforme bovine en Belgique : bilan de l'année 1998. Annales de Médecine Vétérinaire 1999;143:423-436.

41. Centers for Epidemiology and Animal Health (CEAH). National Animal Health Monitoring System. Beef'97, Part II: Reference of 1997 beef cow-calf health and health management practices. United States Department of Agriculture, Fort Collins, 1997, 38 pages.

42. White M.E. Consultant. A diagnostic support system for veterinary medicine. Cornell College of Veterinary Medecine (www.vet.cornell.edu/consultant/consult.asp accessed on 13 November 2011).

Figures and tables

Figure 1. Flowchart of the CART approach with implication of veterinarians (on the left: process; on the right: actors involved)

Figure 2. Classification and regression tree modelling for clinically suspected bovine spongiform encephalopathy cases in Belgium (10)

Legend: BSE, bovine spongiform encephalopathy; LIS, listeriosis; Score, number of clinical signs that are present.

Table I. Bluetongue standardized clinical form for the use in different species (36)

Table II. Variable importance in CART analysis during the first seven weeks of cattle naturally infected by BTV-8 (19)

371 **Table I.** Bluetongue standardized clinical form for the use in different species (36)

General information: Identification number of the herd; Identification number of animal; Animal species; Breed; Sex; Date of birth; Date of last calving; Stage of pregnancy; Date of clinical examination; Name of clinician.

General clinical signs: Hyperthermia; Decreased milk production; Wasting, emaciation, weight loss; Tiredness; Oedema of head, ears, sub-mandibular region, or the peri-orbital region; Hypertrophied lymph nodes.

Clinical signs of skin and annexes: Lesions of the muzzle, lips (congestion > ulcers > necrosis); Conjunctivitis, tears, peri-ocular dermatitis; Photosensibilisation-like lesions; Presence of petechias, contusions, ecchymoses; Erythema, inflammation of the skin, crusts; Cyanosis of the skin or limbs; Skin lesion of the udder, teats or vulva; Scrotal skin lesions; Wool loss (sheep).

Clinical loco-motor signs (musculo-artho-skeletal): Incapacity to lift up or prostration; Reluctance to move or limited movement; Lameness, stiffness of front limbs; Lameness, stiffness of hind limbs; Oedema of coronary bands; Swelling of pastern, fetlock, cannon, carpal or hock joint; Pododermatitis; Contracture of front limbs; Contracture of hind limbs; Arched back; Amyotrophy; Torticollis or neck bended.

Digestive clinical signs: Loss of appetite; Anorexia; Difficulties in grasping the food; Regurgitation; Congestion, erythema of the oral mucosa; Ulcerative lesions of the oral mucosa, excoriations; Salivation, drooling, foam out of the mouth; Oedema and/or protrusion of the tongue; Cyanosis of the tongue; Haemorrhagic stool; Diarrhoea.

Respiratory clinical signs: Ulcerative lesions of the nasal mucosa; Purulent nasal discharge; Mucous, serous, aqueous nasal discharge; Halitosis or bad breath; Dyspnoea, oral breathing, stridor.

Neurological clinical signs: Apathy, lethargy; Generalised weakness, paresis or paralysis.

Reproductive clinical signs: Anoestrus; Abortion or premature calving; Stillbirth; Abnormalities of newborns.

Duration of evolution: Date of the first clinical signs; Comments on the evolution of the disease within the herd.

Post-mortem (PM): Has a PM examination been performed?; If « yes », please attach a copy of the PM record(s) (with the animals identification mentioned).

Concomitant pathologie(s)

372

373

374 **Table II.** Variable importance in CART analysis during the first seven weeks of cattle

375 naturally infected by BTV-8 (19)

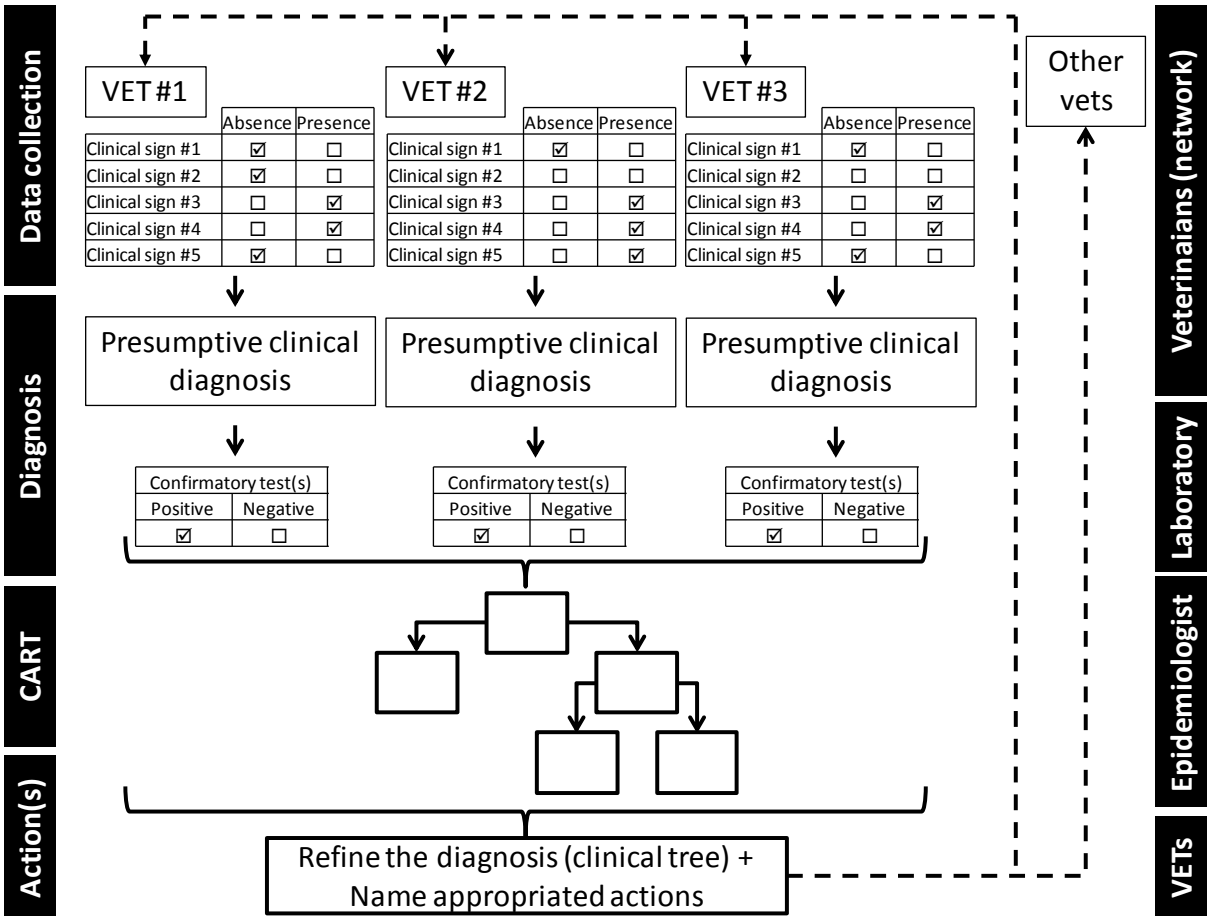
	Variable importance						
Clinical sign	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Conjunctivitis, lacrimation, peri-ocular dermatitis	100	38		33		100	
Ulcerative lesions of nasal mucosa, crusts	32	100	100	91			76
Mucous, serous, aqueous nasal discharge	26	1		100		28	
Congestion, erythema, redness of buccal mucosa and/or muzzle	21			19		61	
Loss of appetite	18		71	18	3	28	27
Purulent nasal discharge	14			6		13	10
Ulcerative lesions of buccal mucosa, excoriation	11		24	44	0		0
Swelling of coronary bands	7					62	
Skin lesions of udder, teat or vulva	1			9	32	18	
Swelling of the head, tongue, sub-maxillary area, jaws			18	22		16	
Lameness or generalised stiffness				2		5	3
Incapacity to stand up, prostration			2	1			3
Anorexia				6			
Tiredness, limited walking				2		47	
Salivation, ptyalism, mouth foam				6		7	
Weight loss			3	62	100	5	41
Arching of back			3				
Muscular atrophia			9	36			
Anoestrus				53		9	5
Milk loss				34	69	78	100
Dyspnoea, buccal breathing, loud breathing				5		19	

376

377

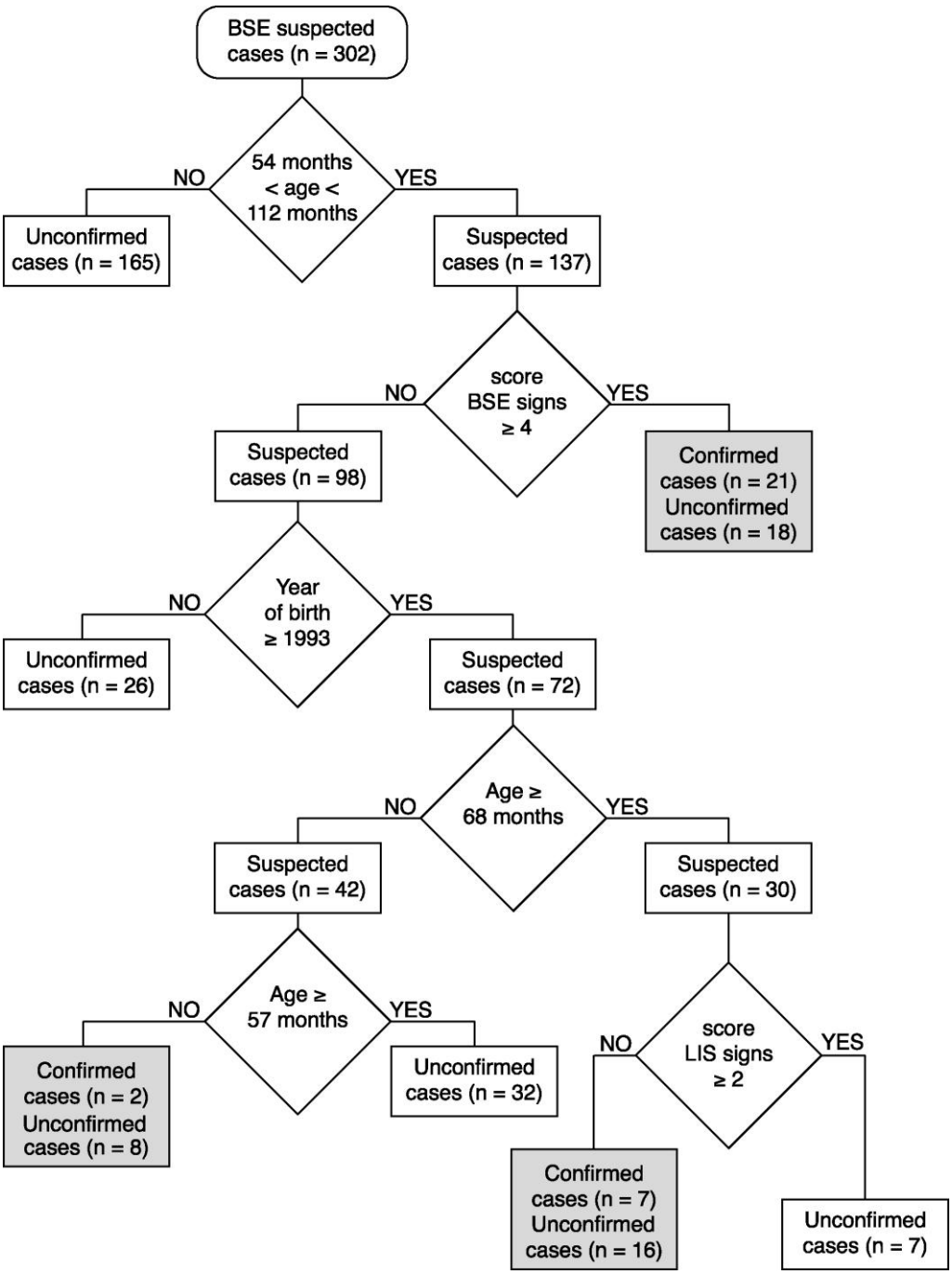
378 **Figure 1.**

379



380

381 **Figure 2.**



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