Epidémiol. santé anim., 1997, 31-32

PREDICTING THE CHANCE OF BVD POSITIVE CULTURES ON CALVES SUBMITTED FOR NECROPSY IN THE NETHERLANDS

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Un protocole standard a été utilisé pour l'examen post-mortem de 1847 veaux au cours d'une période de 3,5 années. En plus des examens pathologiques, bactériologiques et histologiques du cerveau, foie, poumon, rein et intestin grèle, des procédures d'isolement (en double) du virus de la maladie des muqueuses (MMV) ont été réalisées à partir des tissus lymphatiques et de 4 localisations dans le cerveau. Les animaux pour lesquels le MMV a été isolé à partir d'un ou de plusieurs tissus ont été considérés positifs. Le virus a été isolé chez 264 (14,3%) des animaux examinés. Parmi ces animaux, seulement 36 (13,7%) ont été diagnostiqués par le pathologiste comme ayant souffert de MM sur la base des lésions pathologiques qui sont souvent la raison pour réaliser un test de diagnostic MM. Les odds ratios non ajustés à l'autopsie pour quelques lésions pathologiques et/ou diagnoses bactériologiques ont été calculés. Les odds ratios les plus élevés ont été trouvé pour la pneumonie fibrineuse (3,05), l'entérite nécrotique (2,39), les ulcères intestinaux simples ou multiples (4,38), les abcès pulmonaires (12,8), la MM (62,3) et les infections salmonelliques (2,11). Le nombre de procédures d'isolement viral réalisées auraient pu être réduit, tout en accroissant seulement de peu le nombre de résultats faux négatifs, en rejetant les animaux ayant donné les résultats pathologiques et bactériologiques suivants : K99 E. coli-sepsis, pathologie pulmonaire BRSV , empoisonnement par le plomb, traumatisme, défectuosités congénitales des coronaires, perforations intestinales, torsions et obstructions. Le virus de la MM a été isolé le plus souvent à partir de veaux de moins de 8 semaines ayant des lésions pulmonaires, alors que les autres veaux semblaient avoir plus de lésions entéritiques nécrotiques. Les données ont été divisées en fonction des groupes d'âge dans le cadre de 4 analyses de régression logistique. Les modèles finaux ont montré des odds ratios pour l'entérite nécrotique (8,66), la pneumonie fibrineuse (>=2,86), MM (>=44,61), les abcès pulmonaires (>=10,32), les ulcères simples ou multiples (5,76). Il a été conclu que la sélection préalable des cas pour l'isolement viral de la MM sur la base des lésions pathologiques et/ou bactériologiques est une possibilité réelle.

INTRODUCTION

Diagnosis of persistently infected (PI) calves at necropsy is one of the primary diagnostic tools for identification of farms with PI animals (1). The workup for viral isolation on necropsies can exhaust materials and labour for diagnostic labs. However, these laboratories have an important role for determining the presence of BVD circulation in the herd. The purpose of this study was to determine the predictive value of a several protocollaire screening procedures based on gross pathology and bacteriology before virus isolation was attempted.

MATERIAL AND METHODS

From 1988 to 1991 1847 animals submitted for necropsy at the pathology unit were submitted to a standard diagnostic protocol which included the following: Gross pathology, bacteriological analysis and histological examination of brain, lever, lung, kidney and small intestine. In addition to this standard necropsy exam, samples of approximately 50 g were taken from the tonsils (or in their place the mandibular and retropharyngeal lymph nodes), the Peyer's plate cranial to the caecum, the medulla oblongata, cerebellum, lobus ventralis and the corpora quadrigemina, liver, spleen and kidney were used for virus isolation procedures. Samples (duplo) from organs were inoculated on a monolayer of Embryonic Bovine Tracheal (EBT) cells according to standard methods. When BVD virus was isolated from any of the 3 duplo samples, isolation was considered positive for that animal. History on the animals was often limited in nature and in 121 of 1847 missing. The pathologist estimated the age in weeks for those animals for which a history was missing.

STATISTICAL ANALYSIS

Data from the history and gross pathology were coded and entered into a spreadsheet (Lotus Corporation). The data was controlled for errors and the file was read into a statistical program (SPSS Inc, Chicago USA). Crude odds ratios were calculated by dividing the ratio of BVD+/BVD- within a particular subset of gross pathological or bacteriological findings by the ratio of BVD+/BVD- for all cases. Correlation and cross tabulation procedures were used to assess collinearity. The object of the study was not to create a best-fitted model, but to determine which criteria should be used in a rule-in, rule-out protocol for the pathologist to limit the number samples for BVD culture. Using cross tabulation, a number of rule outs were created based on history, gross pathological changes or bacteriological findings. The odds ratio (ratio of BVD positive to BVD negative culture in the group with the particular gross pathology and/or bacteriology, divided by the same ratio of the remaining samples) was used to determine rule outs. Predictor variables which were not highly correlated to each other were placed into the model. Factors were removed from the model in a stepwise manner, based on the significance level and

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possible interaction. Four models were created for four separate age groups, 0-8 weeks (AGE1), 8-24 weeks (AGE2), 24-48 weeks (AGE3),and > 48 weeks (AGE4). For 21 animals there was no age determined. The model produced was by a manual backward step procedure where the criteria were: no. of positive BVD cultures to be maximized while maintaining an overall positive prediction rate of at least 80%.

RESULTS

BVD virus was isolated from 264 (14.3%) of the 1847 animals submitted to necropsy. Of these animals, only 36 (13.7%) were diagnosed by the pathologist as mucosal disease (MD) on the basis of gross pathology. Table II portrays the number of BVD positive and negative animals with various gross pathological and bacteriological findings for all animals examined. The BVD positive animals were not equally distributed among the four age groups. Animals tested positive per age group were: 69/777 (AGE1); 105/578 (AGE2); 43/300 (AGE3); 43/171 (AGE4). Table I shows the correlation between (0,1) factors used in the logistic models.

Table I
Correlation Matrix for variables used in the models

Correlation	ABCES	MDPATH	ENTNEC	PNCAT	PNFIB	ULC12
ABCES	1.0000	.0055	-.0274	.1042**	.0862**	.0243
MDPATH	.0055	1.0000	.0671*	.0491	.0122	4547**
ENTNEC	-.0274	.0671*	1.0000	-.0533	-.0733*	.0084
PNCAT	.1042**	.0491	-.0533	1.0000	-.1722**	.0173
PNFIB	.0862**	.0122	-.0733*	-.1722**	1.0000	-.0115
ULC12	.0243	.4547**	.0084	.0173	-.0115	1.0000

No. of cases: 1475 2-tailed Signif: * - .01 ** - .001

Table II
Categories examined in cross tables as (crude) odds ratios. The categories are not mutually exclusive.
Odds ratio = (BVD+/BVD- for cases of category$_{a..sd}$)/(BVD+/BVD- all other cases)

Findings at necropsy (odds ratio; 95% CI[!])	virus + n	virs - n	Total n
history of poor appetite (1.38; 0.973 - 1.96)	46	210	256
fibrinous pneumonia (3.05; 2.28 - 4.09) PNFIB	89	226	315
catarrhale pneumonia (1.69; 1.16 - 2.47)	40	151	191
bloody enteritis (0.850; 0.555 - 1.30)	27	187	214
necrotic enteritis (2.39; 1.60 - 3.57) ENTNEC	37	101	138
catarrhale enteritis (0.929; 0.714 - 1.21)	111	694	805
navel infection (0.244; 0.059 - 1.01)	2	48	50
one or more intestinal ulcers (4.38; 3.00 - 6.39) ULC12	51	82	133
polyserositis (0.436; 0.156 - 1.21)	4	54	58
encephalitis (1.20; 0.748 - 1.94)	22	111	133
no gross abnormalities (0.443; 0.136 - 1.44)	3	40	43
K99- E. Coli enteritis (0.132; 0.032 - 0.543)	2	86	88
S. dublin or S. typhimurium (2.11; 0.979 - 4.56)	9	26	35
abcesses in the lungs (12.8; 5.96 - 27.8) ABCESS	20	10	30
lead poisoning (0.119; 0.016 - 0.866)	1	49	50
rumen indigestion (< 0.01; -)	0	37	37
torsions, perforations etc. (0.287; 0.69 - 1.19)	2	41	43
other intoxications (0.665; 0.83 - 5.27)	1	9	10
poor body condition (1.92; 1.45 - 2.56)	159	745	904
BLV[#], tumors (1.50; 0.167 - 13.4)	1	4	5
swollen liver, kidney, spleen-(0.286; 0.139 - 0.589) SEPSIS	8	156	164
birth defects, trauma etc. (0.094; 0.013 - 0.687)	1	61	62
lung & intestinal parasites (0.898; 0.265 - 3.044)	3	20	23
(MD)gross pathology (62.3; 21.9 - 176) MDPATH	36	4	40
BRSV pathology in lungs (<0.01; -)	0	42	42
IBR isolation from lungs (2.02; 0.853 - 4.81)	7	21	28
P. multocida in lung tissue (5.56; 3.38 - 9.14)	31	37	68
P. haemolytica in lung tissue (2.06; 1.43 - 2.98)	44	140	184
history of respiratory disease (1.84; 1.35 - 2.53)	63	230	293

[!]Confidence interval, [*] intestinal, [#]Bovine Leukemia

MD pathology was highly correlated with one or more mucosal lesions in the GI tract. MD was defined as more than one typical necrotic ulcera , whereas ULC12 also included animals with a single lesion in the abomasum or elsewhere. Although fibrinous pneumonia and was significantly correlated with mucosal disease pathology, these two terms were allowed in the model because they are different organ systems. This correlation caused MDPATH to loose significance, but it was deemed a critical to the pathological picture and was therefore forced into the models. A number of rule outs were made according to the following criteria: odds ratio less than 0.3 and 95% CI not including 1.0 or bvd+ was zero for the group. The rule outs were: BRSV patholgy in lungs, birth

defects, trauma (including asfyxia), swollen organs, torsions, perforations (including ruptuur) of the intestines, rumen indigestion, lead poisoning, k99-E.Coli. In total, 392 cases were removed from the data set including 20 cases for which no age was determined. Of these 392 cases, only 8 were positive for BVD . For the remaining 1255 samples, logistic models gave the following predictions.

Table III

Model for AGE1: Logit(BVD) = 1.61 (abcess in lungs) + -2.09 (sepsis) + 1.05 (fibrinous p neumonia) -2.14 (constant Predictions AGE1)

Variable	B	S.E.	Wald	df	Sig.	
Constant	-2.138	.1522	197.3	1	.0000	
SEPSIS	-2.090	1.015	4.235	1	.0396	1.352
PNFIB	1.051	.3465	9.200	1	.0024	3.05
ABCESS	1.612	.8629	3.491	1	.0617	12.8

Using a cutoff point for prediction of 0.0144 instead of 0.5 (normally used for outcome prediction) was equivalent to testing all animals with any one or more of the following: no sepsis, fibrous pneumonia or an abces in de lungs. There were 582 cases and 65 BVD positive animals (64 predicted) in this group The results, at the 95% confidence level, are shown below. Similar procedures were repeated for the other age groups.

Table IV

Model for AGE2: Logit(positive BVD) = 2.33 (abcess in lung) + .8.62 (MD -gross path) + 1.26 (fibr. pneumonia) - 1.93 (constant)

Variable	B	S.E.	Wald	df	Sig.	ODDS
constant	-1.933	.1636	139.6	1	.0000	
ABCESS	2.335	.6352	13.51	1	.0002	10.32
PNFIB	1.267	.2447	26.85	1	.0000	3.55
MD-PATH	8.629	12.57	.4707	1	.4927	5593

There were 484 cases of which 103 were BVD positive for AGE2.

Table V

Model for AGE3: Logit(BVD)= 3.79 (MD gross path) + 2.64(abcess in lung) + .816 (fibr. or catarrhal pneumonia) -2.41(constant)

Variable	B	S.E.	Wald	df	Sig.	ODDS
constant	-2.41	.3087	61.13	1	.0000	
ABCESS	2.645	.8776	9.08	1	.0026	14.08
PNTOT	.8164	.4039	4.08	1	.043	2.262
MD-PATH	3.797	.8098	21.99	1	.0000	44.61

There were 242 cases of which 52 were BVD positive in AGE3

Table VI

Model for AGE4: Logit(BVD)= 2.158 (necrotic enteritis) + 1.752(Ulcers in one or more areas of the enteric mucosae) -1.67(constant)

Variable	B	S.E.	Wald	df	Sig.	ODDS
constant	-1.672	.2725	37.67	1	.0000	
ENTNEC	2.158	.6783	10.13	1	.0015	8.66
ULC12	1.751	.4289	16.68	1	.0000	5.76

There were 147 cases of which 52 also were BVD positive.

DISCUSSION

Although bacteriology and history were not involved in our final models, the crude odds ratios for salmonella, and P. multocida support the findings of Penny (2).The most important predictors for positive culture were ULC12, ABCESS and PNFIB. Pneumonia seemed to play a greater role in the age groups below 48 weeks, whereas few young calves sufferred from MD. A. substantial reduction in the number of tests done can be accomplished by a simple rule out protocols. In our study, the prevalence ratio in the animals submitted for BVD testing increased from 14.3 to >20 % by this method. For the remaining 1455 animals, 401 animals would have been tested and 171 animals found using the presence of abscence of factors in the model as the decision criteria. However, 119 animals would have been missed (false negatives) using the same protocol, because of a low sensitivity (except for AGE1).

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