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| **Supplementary figures**   |  | | --- | |  | |

Fig. S1. ROC curves (dotted line) for SPLA (A), rK39 (B), SECA (C), SPLA/SECA (D), rK39/SECA (E) and rK39/SPLA (F). Identity is represented by the solid line. The data depicted was generated from the two independent assays performed in triplicate

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Fig. S2. Reactivity of dog sera from individual countries against rK39 (A) and SPLA (B) antigens: Hungary (HU), the Netherlands (NL), the United Kingdom (UK), Germany (DE), France (FR), Denmark (DK) and Poland (PL). Results are expressed as the logarithm of the optical density (OD) at 492 nm normalized by the cut-off value for each antigen (n). Bars represent the median for the cohort and each single dot represents the data set for an individual dog. The data depicted was generated from the average of at least two independent assays done in triplicate.

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Fig. S3. Relationship between different relative antigen reactivity in the control cohorts. Graphical representation of the logarithm of the relationship between the normalized optical densities (at 492 nm) from selected pairs of antigens. (A), rK39/sPLA vs SPLA/SECA; (B) rK39/SPLA vs SPLA/SECA; (C) rK39/SPLA vs rK39/SECA. Each data point is labeled with the unique experimental ID. The data depicted was generated from the average of at least two independent assays done in triplicate.

SPLA vs SECA(A) and rK39 vs SECA (B) for control CanL+ and CanL- cohorts. Each dot represents a serum sample from an individual dog.

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| C:\Lima\Desktop\10 November\Figures Tables 2016 11 22\Supplementary Material 2016 11 22\Supplementary figures 600dpi\Figure S4.tif  Fig. S4. Influence of cut-of stringency in seropositivity. Graphical presentation of the seroreactivity to rK39 and SPLA as the optical density (OD) at 492 nm normalized by the cut-off value for each antigen (n). Dashed lines represent the number of cut-offs (n x cut-off) for each antigen. The data depicted was generated from the average of at least two independent assays done in triplicate.  Representation of seroreactivity against SPLA and rK39 antigens in European cohort in function of the number of cut offs (vertical dotted lines). Score value is represented for each sample. Results are expressed as the optical density (OD) at 492 nm normalized by the cut-off value for each antigen (n) and each single dot represents a serum from an individual dog. |