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Comments on Performance and Interpreting the Cornell Kinetics ELISA (KELA)

The KELA value is a measure of the amount of *Mycobacterium paratuberculosis* antibody detected in the sample, normalized to a set of standards. This test is intended primarily for herd testing and profiling antibody responses within a herd or group of animals. However, test results for individual animals may be used and interpreted by the following criteria:

KELA ELISA units of:

- 0-39 = Lowest risk of infection
- 40-54 = Moderate risk of infection
- 55-164 = Moderately high risk of infection
- >164 = High risk of infection

It is important to take into account status of infection in the herd and age of the individual in interpreting ELISA results in an individual. In general, ELISA can suggest a risk of being infected but in itself cannot confirm the presence or absence of infection. This test is best used in decision making in conjunction with other information. Herd and individual animal history and confirmatory tests such as the fecal culture must be used to augment the results from the ELISA in making herd and individual animal decisions. This is due to the fact that all herds are not the same, in terms of management, health, stress, environment, genetics and other factors that could influence the biology of *M. paratuberculosis* infection or antibody responses in an ELISA test system. Individual animals, as well as herds, can display antibody responses on an ELISA that can be either falsely elevated or not elevated though infected.

Interpreting KELA ELISA values in the context of the herd

Lowest risk

Animals having KELA activity between 0 and 39 units are at the lowest risk of infection. The value 39 is approximately 2X the mean KELA value in non-infected NY herds used in the validation. Because antibody is not usually detected early in Johne's infections, animals may be classified as having the lowest risk of infection, but nevertheless be carrying the organism (the inherent sensitivity of all ELISAs is low because antibody is a relatively late response in this infection). This is particularly true of most infected heifers (too young to have any antibody response) and over 50% of individual infected cattle from known infected herds.

However, on a herd basis, if > 95% of adult cows in a herd have values in this category, in conjunction with no history of disease, there is a high probability that the herd is not infected, or the prevalence is low. One potential caveat (anecdotal) is the possibility that Johne's infection may be masked in a herd (no clinical disease and perhaps low antibody responses) if management and nutrition are consistent and adequate, i.e. animals consistently managed, adequate nutrition, healthy and relatively unstressed. Stress, however, may cause the infection in a herd or individual to become manifest.

Moderate risk

Animals with KELA activity in this category (40-54) would have a minimal chance of being infected if they came from a herd that had no previous history of Johne's disease. Alternatively, if the herd from which the animal came has had a history of Johne's, chances are slightly increased that the elevation in antibody is due to infection. This KELA value, even in an infected herd, however, only suggests that the risk may be relatively higher, and in no way confirms presence or lack of infection.

A value of 55-60 is approximately 3X the mean KELA value in the non-infected validation herds. In some herds, values such as these could also be due to cross reactivity to some other agent in the herd. It is not possible to know based on a single test in an individual.

Before making culling or grouping decisions on animals in this category, they should be evaluated for shedding status by fecal culture or monitored and retested in 6-12 months. This degree of elevation however can be used to make management decisions that have a high return if the risk is correct and a low cost if it is in error, such as not using colostrum or using with other criteria in making purchase decisions.

Moderately high risk – a broad category

This category is very large (55-164) and ranges from 3 to 10X the mean KELA value in non-infected herds. It is more useful to look at lower and higher values within the category: the 55 – 90 (80-100) range and 90 – 164 ranges.

Values of 80 - 100 span the 98-99% specificity level, which would be equivalent to the range chosen for a single cut-off interpretation if that was used. The relative sensitivity of the KELA at values of 80 -100 is approximately 40-50% based on validation in animals in herds and determined infected by fecal culture. A sensitivity of 25-30% is a more functional estimate of the overall performance of Johne's serology. This is the same estimate recommended for Johne's ELISA serology by the National Johne's Working Group.

Interpretation in known infected herds

In general, animals having ELISA values in this broad category, that come from a herd with known Johne's infection have a significant probability that antibody is the result of infection. Knowing the animal is from an infected herd is important in interpreting individual responses as it increases the probability that a positive test result is correct. If infected animals are known to exist in the herd, the chance that any individual is infected increases (compared to individuals in herds with no or low infection). There is also an increasing chance that an animal is infected as the ELISA value approaches the upper end of this category i.e. 90 units or above. These two pieces of information combined contribute to an animal with an ELISA in this MH category having a higher chance of being infected. For values above 80-100, the chance of infection is very high. In general in infected herds, the risk is higher the higher the KELA value.

One caution and disadvantage of the ELISA is that some individuals in advanced infection do not have elevated antibody for reasons unknown. Approximately 10-15% of high-risk animals, Many shedders on fecal culture, do not have KELA values above 80. Approximately 80% of Moderate fecal shedders will not have KELA values above 80.

Interpretation in non-infected herds – “false positive” responses

In contrast to infected herds, 2% of animals in a non-infected herd can be expected to have a “false-positive” elevated KELA value greater than 80-100. For the Cornell KELA ELISA, 80-100 spans the range from 98 to 99% specificity. This means that in non-infected

animals, 98% will have a KELA value less than 80 (2% will have a value greater than 80). This was determined by validation in known non-infected NY dairy and beef herds.

A KELA of 80-90 is analogous to a single cut-off point above which all animals could be considered “positive” and below which they would be considered “negative”. A single cut-off point is typically used in other commercial ELISA tests for keeping interpretation both simple and optimally accurate. For Johne’s ELISA tests, the goal is to choose a cut-off that maximizes accuracy and minimizes false positives. The cut-off often used is one above which only 2% of non-infected animals would be expected to test positive (false). The disadvantages of the single cut-off test are that it does not reflect the actual biology of Johne’s disease and much information about the possible infection status of the animal is lost.

The percent of animals in a non-infected herd with “falsely positive responses” will increase as the value of a cut-off is moved downward. For example, 3-4% of non-infected animals would be expected to have a KELA greater than 75, and 10% greater than 65 and so on. Thus, even in a non-infected herd, 0-3 animals out of 100 might be expected to have “elevated” KELA values >80. The percent will vary herd to herd depending on the extent of background activity and exposure to cross-reacting agents.

In a herd estimated to have no or a low level of infection, the elevated KELA has a high probability of being a false positive result.

What is the chance the positive result is correct?

In herds with established infection, the chance that an elevated ELISA (80-100+) is correct is quite high, and increases with the prevalence in the herd. Another way of putting it is that the predictive value of a positive result in an individual, or the chance that it is correct, is greater the higher the prevalence in the herd.

To illustrate, take an infected herd with a prevalence of 20%, which is not uncommon. The number or proportion of infected animals that have an elevated ELISA of 90 or higher might be 30% (Sensitivity of the KELA) of the 20%, or 7% of the herd. Also recognize that non-infected animals in infected herds have false positive responses at a similar rate to non-infected animals in non-infected herds (i.e. 1-3%). However, when infection is present in the herd, the proportion of true positives is often much greater than the number or proportion of animals that have falsely elevated responses. In this case % of the 70% non-infected or 1.4% of the herd might be expected to have elevated values. The elevated value has about an 80% chance of being correct, or a predictive value of about 80%.

On the other hand, in herds with very low infection (i.e. 5% or less) there is a good or equal chance that an elevated ELISA result (80-100 range) is false, or the predictive value of the positive result is poor. In such a herd, 2/95 (2%) non-infected animals will be false “positive”, and at best 1-2 (30%) of the 5 infected animals will be true “positive” (> 80), or 1-2% of the herd. The chance that an KELA value >80 in this herd is falsely elevated can be higher than the chance that it is due to the animal truly being infected. In this case the predictive value of the positive result 30 to 50% at best.

“False negative” results

Many infected animals in an infected herd may have elevated values that are greater than 54 and less than 80 – 100, and should be regarded as having a higher risk of being infected. However, similar to animals in the MODERATE risk category, other information about the individual, the herd and repeated testing should be used to make decisions.

In infected herds, when owner/veterinarian gain experience from using the test and from repeated testing on individuals, KELA test results become more “meaningful”. Ability to interpret and apply test results to accomplish herd objectives improves with experience, as more is learned about the prevalence, exposure risks and from changes and outcomes of results over time.

High risk – KELA values greater than 130

This category (>165) is extremely high and not particularly useful. KELA values greater than the 130 range (clinical experience) can be considered high risk. Animals in these ranges are most likely infected. The probability is near certainty if animals come from a known infected herd or the individual has clinical signs of Johne’s disease.

On a herd basis, herds with established infection on testing will have 1-2% of animals in the high (>164) category. Herds with high prevalence infection may have 3-4%, but this is uncommon. Infected herds with control programs with a moderately effective test and cull component will no longer find animals in this category. Although possible, values this high are unusual non-infected herds, and status should be further investigated.

Test results should concur with expectations

Test results should concur with what you expect based on the history and conditions. The other information and testing is as important, and often more important, to support, or question, the probabilities suggested by tests.

Fecal culture should be used further evaluate status

The routine use of fecal culture to obtain more definitive evidence of infection and shedding in the herd, individuals with elevated serology results, whenever Johne’s status influences significant animal or management decisions is strongly encouraged. Much more is learned by having the information from both tests. It is important to note that despite the fact that fecal culture is a more accurate test, a significant proportion (>50%) of cattle are likely to culture negative in face of an “elevated” ELISA value. Which factors play the greatest role: biologic, shedding, antibody, sampling, or test is not known. Repeat testing and other information is needed to further clarify the status of these individuals.

History is essential in interpreting individual ELISA values – Predictive value of results

Herd history and prevalence

Knowledge about the history of Johne’s in the herd is essential to improve interpretation of a KELA ELISA result in individuals in the herd. This is because the true proportion of infected animals in a herd influences the likelihood that a value obtained in one individual from the herd correctly indicates the individual’s infection status.

It is important to have an expectation or estimate of the prevalence of infection in the herd based on history i.e. number and age of clinical cases, imported animals, extent of risks for spread, other testing, etc. The expected prevalence should then be taken into account when interpreting the likelihood that an antibody level in an individual in the herd, measured by the ELISA, is an accurate indicator of infection status. For the same KELA value, the chance that the value correctly indicates infection or not in a particular individual will vary with the prevalence of infected animals in the herd or group the individual originates from.

Sensitivity, specificity, prevalence, and predictive value

Sensitivity of an ELISA test refers to the proportion of known infected animals expected to test positive on the test. Specificity is the proportion of known non-infected animals expected

to negative on the test. These parameters are determined in the process of evaluating how the test performs in known infected (sensitivity) and non-infected (specificity) populations of animals. However, herds in which the test is eventually used are often made up of a mix of infected and non-infected animals. Knowing what the mix might be helps interpret the probability that a positive or negative result on an animal from that herd is correct or not. The prevalence is an estimate of the mix, or of the percent of infected animals in (or that might be) the herd.

The chance or probability that a particular test result is correct is a valid measure of test performance, which can be calculated for positive and negative test results. It is based on the existing estimated prevalence in the animals being tested and the sensitivity and specificity of the test being used. It is called the predictive value of a positive or a negative test result.

The following principles generally apply when interpreting Johne's serology- taking into account a guesstimate of the prevalence (probability) of infection in the animals being tested.

Predictive values in a herd with known higher prevalence of infection

The chance that an elevated KELA value (positive predictive value) in an individual from this herd accurately reflects infection is quite high. This is because there are a high proportion of infected animals in the herd to produce a positive test result.

On the other hand, the chance that a KELA value in the LOW range (negative predictive value) correctly indicates lack of infection in an individual in this herd is quite low. This is because potentially there are a high number of infected animals in the herd, and a high proportion of those (100% - a Sensitivity of 30% = 70%) are expected to have false negative test results.

Predictive values in a herd expected to have no or low prevalence of Johne's infection

The chance that a KELA value in the LOW category in an individual in this herd correctly indicates lack of infection (negative predictive value) is quite high. This is because based on the herd history, the number of infected animals and thus the number that could test falsely negative is low compared to the high proportion of non-infected animals in the herd that test truly negative.

Similarly, the chance that an elevated KELA value (i.e. in the MOD-HIGH range) in an individual in this herd correctly reflects true infection (positive predictive value) is quite low. This is because when there are no or very few true infected animals in a herd, the probability that an elevated KELA in an individual is a false positive response becomes very high.

Predictive value in a herd of unknown status, without a the context of a history of probability of infection

It should be easier to now understand why without a prior indication of the risk of infection in a herd or the individual it is difficult, if not impossible to interpret an individual Johne's ELISA result. When using imperfect diagnostic tests such as the Johne's ELISA, prior information about the probability of infection is necessary to help "stack the deck" in interpreting results. That information is crucial in order to improve one's estimate of the probability that an elevated result is either a true or false response, or that a low result suggests lack of infection or just a falsely negative response in an infected animal.

For more information on predictive value and interpreting Johne's test results

For more discussion on predictive values and interpreting Johne's test results see NYSCHAP Articles 4 through 7 on Johne's disease tests, and interpreting Johne's disease test results.