Pulpy kidney disease or enterotoxaemia is an acute disease of sheep which leads to sudden death. In practice clinical cases are seldom seen. The occasional cases that are seen display nervous symptoms and sometimes slight diarrhoea. At post-mortem no signs pathognomonic of this disease can be detected. Since these circumstances readily lead to confusion in diagnosis for the field worker it seems appropriate to consider the different aids that can be applied when in difficulty. Before, however, discussing the criteria for establishing a diagnosis individually, an account will be given of the pathogenesis of pulpy kidney disease as it is generally understood at present:

Bennetts detected Cl. welchii type D from the abomasum of two healthy sheep in an area where the disease had occurred. Bullen detected the same organism in the alimentary contents of 46 of 100 normal sheep killed at the Cambridge abattoirs. It is, therefore, well established that the causal organism can be present in the intestinal contents of normal sheep and it seems likely that the disease develops when conditions in the intestines become suitable for its multiplication and toxin production.

Bullen and Batty give an account of the development of a method of experimental reproduction of enterotoxaemia and mention the following findings:

Continuous dripping of actively growing Cl. welchii type D culture into the duodenum of normal sheep for hours does not produce the disease as long as the sheep are fed on a normal diet. The disease can, however, be reproduced if the diet of the sheep is suddenly changed to rich food e.g. wheat and they are allowed to over-eat before the culture is dripped into the duodenum. When a digestive upset occurs under these circumstances undigested starch granules escape from the rumen and enter the small intestine. Growth and toxin production of Cl. welchii type D is stimulated by the starch granules leading to an accumulation of bacteria and toxin in the lumen of the intestines. Whereas normally a high concentration of toxin and bacteria is rapidly cleared from the gut by the onset of a diarrhoea, under these circumstances the clearing mechanism cannot cope with the accumulation of toxin.

A low concentration of epsilon toxin in the gut is harmless and remains virtually unabsorbed, but lethal amounts of toxin are rapidly absorbed if high concentrations are maintained in the gut for some time. High concentrations of toxin in the lumen increase the permeability of the gut wall. The absorbed toxin is directly responsible for the death of the sheep.

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The above observations explain the rationale of some of the criteria applied for arriving at a diagnosis. An evaluation of the diagnostic aids follows:

(1) Post mortem appearance:

As a result of the development of a relatively simple method of experimental reproduction of enterotoxaemia, it has been possible to observe the post-mortem appearance of carcases at different stages after death. In a fresh carcase the bowels are invariably distended with gas; the kidneys are swollen, hyperaemic and the pericard is distended with clear fluid which may contain a coagulum. Sub-endocardial and -epicardial petechiae are commonly observed but for the rest there is nothing typical. The carcases tend to undergo putrefaction rather rapidly; the kidneys becoming pulpy.

(2) Detection of epsilon toxin in the gut contents.

In a fresh carcase epsilon toxin can be detected readily in the gut contents and the specificity of this toxin can be proved by conventional methods. In a carcase that was left lying at room temperature for 12 hours toxin could still be detected in the intestinal contents.

The intestinal contents from a fresh carcase were collected in a clean container and preserved by the addition of one drop of chloroform to every 10 ml. of liquid and left standing at room temperature. At irregular intervals its toxicity was tested and the specificity of the toxin proved by serum-neutralisation. It was found that this material was still toxic one month after collection.

When, however, a section of intestine containing toxic contents is tied-off at both ends and preserved in 50 per cent glycerine, the toxicity of the contents is lost within 12 hours.

It, therefore, seems advisable to collect intestinal contents from fresh carcases and preserve this material with chloroform for submission to a laboratory for diagnosis. More success is likely to follow this procedure than submitting sections of intestine in 50 per cent glycerine.

The detection of epsilon toxin in the gut contents of sheep is not conclusive evidence that the animal has died of enterotoxaemia, since immune sheep can tolerate high concentrations of toxin in their intestines.

(3) The presence of Cl. welchii type O in the gut contents.

Since this organism has been isolated from the intestinal contents of normal sheep, finding it in a specimen of gut contents has no diagnostic significance.

(4) The detection of typical Cl. welchii type O in smears of intestinal contents.

Smears should be prepared from the contents of the ileum and stained by Gram's method.

In cases of enterotoxaemia typical Cl. welchii, i.e. short, fat, Gram-positive organisms, occur in the smear to the complete or almost complete exclusion of all else. In my own experience this is a very common finding, confirming the views of Rowlands.
(5) Glycosuria.

Gordon, Stewart, Holman and Taylor\textsuperscript{5} report that urine from cases of enterotoxaemia caused by \textit{Cl. welchii} type D contain a great deal of sugar. The sugar content of the blood in these cases was equally high. This upset of sugar metabolism could not be detected in animals injected with the toxins of \textit{Cl. welchii} types B and C.

Bullen and Batty\textsuperscript{3} found a well-marked hyperglycaemia and an associated glycosuria in 9 out of 10 experimentally produced cases of enterotoxaemia (1 was not examined). They used this symptom to distinguish between enterotoxaemia and plain acidosis following on over-eating.

The occurrence of glycosuria in natural cases of enterotoxaemia was confirmed by me.

(6) Level of immunity.

Smith and Marsh\textsuperscript{6} state: "0.3 units of antitoxin per millilitre suffices to give adequate protection, and it is probable that appreciably lower concentrations are sufficient to give protection against enterotoxaemia".

Thomson and Batty\textsuperscript{7} estimate that an antitoxin level in the order of 0.1 unit per ml. is the lowest level which will afford protection against enterotoxaemia.

Jansen\textsuperscript{4} challenged immune sheep by injecting culture and dextrin into their duodenum. Before being challenged they were bled and the antitoxin value of their sera determined in terms of Wellcome units. The results are recorded in Table 1.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Number challenged} & \textbf{Antitoxin level units ml.} & \textbf{Result} \\
\hline
2 & 0 & Both died \\
7 & 0.1 & 3 lived 4 died \\
4 & 0.15 & 4 lived \\
2 & 0.3 & Both lived \\
\hline
\end{tabular}
\end{table}

From these results it can be reasonably concluded that an antitoxin level of at least 0.15 units per ml. of circulating antitoxin renders a sheep immune to pulpy kidney.

At times veterinarians and farmers complain that sheep keep on dying of enterotoxaemia in spite of repeated injections of alum-precipitated epsilon toxoid at reasonable intervals. In several such cases I have requested the veterinarians in charge of flocks affected
to send me serum samples from sheep selected at random. In every instance I established that the serum samples showed a high antitoxin titre (sometimes as high as a mean value of 30 units per ml.). The significance of this finding is that the sheep concerned were fully protected by the injections of vaccine and, therefore, could not have died of enterotoxaemia.

Jansen⁴ proved that when sheep were given two injections of Onderstepoort Pulpy Kidney Vaccine as primary and secondary stimulus at an interval of 3 to 4 weeks, they showed a geometric mean titre of 42 units per ml. one week after the second dose.

(7) The detection of *Cl. welchii* type D in liver, kidney and lymphnode specimens.

As mentioned under point (3) above, the isolation of *Cl. welchii* type D from intestinal contents has no diagnostic significance. In the cases of enterotoxaemia artificially produced at Onderstepoort⁴ specimens from the liver, kidney and mesenteric lymphnodes were collected at post-mortem with aseptic precautions before the intestines had been opened. Subsequently material from these different organs was transferred separately into Robertson's meat broth tubes and pasteurised at 60°C for 30 minutes. After overnight incubation the contents of the tubes were tested for the presence of epsilon toxin by conventional methods. The results are recorded in Table 2.

From these results it can be seen that out of the 16 liver specimens examined 14 were found positive; all 6 kidney specimens examined were positive, and only 4 out of 16 lymphnode specimens were negative.

Whereas the epsilon toxin is directly responsible for the death of an animal, it seems that the organisms enter the bloodstream in the terminal stages of the disease. While this occurs in the artificially produced cases, there seems to be no reason why it should not take place in the natural disease. Field veterinarians are urged to collect liver and kidney specimens from suspected cases and submit them to the laboratory in 50 per cent glycerine. The specimens should, however, be collected as cleanly as possible before opening the gut.

(8) The occurrence of pulpy kidney after dosing phenothiazine.

Since the introduction of phenothiazine as anthelmintic occasional reports have been received at Onderstepoort of sheep dying of enterotoxaemia subsequent to dosing. No occasion for proving these claims arose until Jansen⁴ produced enterotoxaemia in susceptible sheep by dosing them with the therapeutic dose of phenothiazine and immediately afterwards introducing 20 ml. of actively growing culture of *Cl. welchii* type D into their duodenum under local anaesthesia. Control susceptible sheep, receiving the culture only, lived.

This finding emphasises the necessity of ensuring a protective level of immunity before dosing sheep with phenothiazine.

**Differential Diagnosis**

(1) Overeating predisposes to enterotoxaemia but by itself can be responsible for death in sheep. When sheep overeat on a carbohydrate rich diet they develop an acidosis. This, however, is likely
TABLE 2
THE DISTRIBUTION OF Cl. welchii TYPE D IN ORGANS COLLECTED AT POST MORTEM

<table>
<thead>
<tr>
<th>Sheep No.</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lymph-gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>7815</td>
<td>+</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>8107</td>
<td>+</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>8115</td>
<td>+</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>8125</td>
<td>+</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>7902</td>
<td>+</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td>8114</td>
<td>-</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>8098</td>
<td>+</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>8097</td>
<td>-</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td>8688</td>
<td>+</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>8036</td>
<td>+</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>8053</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8708</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8705</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8069</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8711</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7984</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = positive for Cl. Welchii type D.
- = negative
NT = not tested

to occur only under feedlot conditions where enterotoxaemia is also expected to occur. Bullen and Batty\(^3\) state that enterotoxaemia can be differentiated from acidosis by the fact that hyperglycaemia and glycosuria are not shown by sheep dying of acidosis. This was confirmed by Brown and Jansen\(^8\).

Sheep being fed on a protein rich diet under feedlot conditions may develop an alkalosis due to overeating. Clark\(^9\) established that sheep dying of alkalosis as a result of overdosing with urea show a marked hyperglycaemia and glycosuria. It is furthermore a well established fact that sheep in the terminal stages of certain metabolic diseases e.g. enzootic icterus, domsiekte and geeldikkop show hyperglycaemia and glycosuria.

From this can be concluded that the absence of sugar in the urine collected from a dead sheep has differential diagnostic significance in so far as it points very definitely to the exclusion of enterotoxaemia as the cause of death.
(2) Rowlands\textsuperscript{10} mentions that in North Wales metabolic disorders among sheep associated with improved grassland followed a pattern closely resembling enterotoxaemia.

(3) In South Africa "geilsiekte" which is synonymous with hydrocyanic acid poisoning is very frequently confused with enterotoxaemia.

(4) Fungus intoxication can be responsible for sudden deaths and the active principle contained in some fungi has a severe diuretic effect leading to hyperaemia of the kidneys.

(5) Vegetable poisoning leading to sudden death without typical post mortem lesions.

DISCUSSION

Although the diagnosis of pulpy kidney disease is certainly not easy, it should be possible to reach a decision when the points listed above are considered. In the first instance the flock as a whole or the majority of its members must be susceptible (the susceptibility of sheep can be easily determined by antitoxin titration of the sera of sheep selected at random). There is usually a history of a change of grazing followed by sudden deaths, but sometimes, especially where sheep are kept on rich pasture permanently, a change of grazing is not a prerequisite. The sheep dying of enterotoxaemia show a negative post mortem except for softening and reddening of the kidneys, accumulation of gas in the intestines and hydropericardium. Smears taken from the intestinal contents show typical \textit{Cl. welchii} type D organisms to the exclusion of other bacteria; the presence of epsilon toxin in the gut contents can be demonstrated and the organism can be isolated from kidney and liver specimens.

When, through unforeseen circumstances, it is impossible to obtain fresh carcases for post mortem examination or when it is impossible to reach a conclusion after conducting post mortem examinations, recourse could be taken to vaccinating the flock against pulpy kidney. When sheep which have a basic immunity, i.e. which have had an injection or injections of alum-precipitated pulpy kidney vaccine more than a month previously, receive a booster injection of alum-precipitated pulpy kidney vaccine they should be fully resistant to the disease after about seven days.

SUMMARY

A description of the pathogenesis of enterotoxaemia is given as well as a description of the laboratory and field aids that can be applied to arrive at a diagnosis. The differential diagnosis is discussed.

REFERENCES